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APPLICATION NUMBER: *60/526,797*
FILING DATE: *December 04, 2003*
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Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
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Patent and Trademark Office

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a **PROVISIONAL APPLICATION FOR PATENT** under 37 C.F.R. § 1.53(c).

	Docket Number	21101.0051U1	Type a Plus Sign (+) inside this box	+

INVENTOR(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (City and Either State or Foreign Country)
Prestwich	Glenn	D.	Salt Lake City, Utah
Shu	Xiao	Zheng	Salt Lake City, Utah

TITLE OF INVENTION (500 characters max)

MODIFIED MACROMOLECULES AND METHODS OF MAKING AND USING THEREOF

CORRESPONDENCE ADDRESS

Customer Number 23859

ENCLOSED APPLICATION PARTS (*Check All That Apply*)

- ☒ Provisional Application Title Page — *Number of Pages* [1]
- ☒ Specification (includes Description, Claims, & Abstract) — *Number of Pages* [116]
- ☒ Drawing(s) — *Number of Sheets* [7]
- ☒ Authorization to Treat Reply Requesting Extension of Time as Incorporating Petition for Extension of Time
- ☒ Other (specify): Return Postcard

METHOD PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (*Check One*)	
☒ Applicant claims small entity status. See 37 CFR § 1.27. ☒ A Credit Card Payment Form PTO-2038 is enclosed to cover the filing fees. ☐ A check or money order is enclosed to cover the filing fees. ☐ The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number _____. ☒ The Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 14-0629.	**FILING FEE AMOUNT** $ 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☒ Yes. The name of the U.S. Government agency and the Government contract number are: The National Institute of Health, Grant No. NIH5R01 DC04663

Respectfully submitted,

Signature _____ **Date** December 4, 2003

Typed or Printed Name: Lawrence A. Villanueva

Registration No. 43,968

CERTIFICATE OF EXPRESS MAILING UNDER 37 C.F.R. § 1.10

I hereby certify that this correspondence and any items indicated as attached or included are being deposited with the United States Postal Service as Express Mail, Label No. EL992019977US, in an envelope addressed to: **MAIL STOP PROVISIONAL PATENT APPLICATION**, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

_____ 12/4/03

Michael Laird — Date

ATTORNEY DOCKET NO. 21101.0051U1
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
PRESTWICH, *et al.*	)	Art Unit: Unassigned
	)	
Application No. Unassigned	)	Examiner: Unassigned
	)	
Filing Date: Concurrently	)	Confirmation No. Unassigned
	)	
For: "**MODIFIED MACROMOLECULES AND METHODS OF MAKING AND USING THEREOF**	)	

AUTHORIZATION TO TREAT REPLY REQUIRING EXTENSION OF TIME AS INCORPORATING PETITION FOR EXTENSION OF TIME

MAIL STOP PROVISIONAL PATENT APPLICATION
Commissioner for Patents
P.O. Box 1450
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NEEDLE & ROSENBERG, P.C.
Customer Number 23859

December 4, 2003

Sir:

Pursuant to 37 C.F.R. § 1.136(a)(3), the Commissioner is hereby requested and authorized to treat any concurrent or future reply in the above-identified application, requiring a petition for an extension of time for its timely submission, as incorporating a petition for extension of time for the appropriate length of time.

The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

NEEDLE & ROSENBERG, P.C.

Lawrence A. Villanueva
Registration No. 43,968

NEEDLE & ROSENBERG, P.C.
Customer No. 23859
(678) 420-9300
(678) 420-9301 FACSIMILE

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Michael Laird — 12/4/03 (Date)

Express Mail No. EL992019977US
Attorney Docket No. 21101.0051U1
PATENT

PROVISIONAL APPLICATION
FOR
UNITED STATES LETTERS PATENT
FOR

MODIFIED MACROMOLECULES AND METHODS OF MAKING AND USING THEREOF

BY

GLENN D. PRESTWICH, a citizen of the United States of America, residing at 1500 Sunnydale Lane, Salt Lake City, Utah 84108; and XIAO ZHENG SHU, a citizen of China, residing at 25 South 1100 East, No. 15, Salt Lake City, Utah 84102.

MODIFIED MACROMOLECULES AND METHODS OF MAKING AND USING THEREOF

ACKNOWLEDGEMENTS

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BACKGROUND

The use of macromolecules in pharmaceutical applications has received considerable attention. At times, it is desirable to couple two or more macromolecules to produce new macromolecule scaffolds with multiple activities. Existing technologies used to couple two or macromolecules, however, present numerous difficulties. For example, the alkaline conditions or high temperatures necessary to create hydrogels with high mechanical strength are cumbersome and harsh. Although the use of crosslinkers to produce macromolecular scaffolds has met with some success, the crosslinking agents are often relatively small, cytotoxic molecules, and the resulting scaffold has to be extracted or washed extensively to remove traces of unreacted reagents and byproducts (Hennink, W. E.; van Nostrum, C. F. *Adv. Drug Del. Rev.* **2002**, *54*, 13-36), thus precluding use in many medical applications. A physiologically compatible macromolecular scaffold capable of being produced in a straightforward manner is needed before they will be useful as therapeutic aids. Described herein are compounds and methods that are capable of coupling two or more molecules, such as macromolecules, under mild conditions.

SUMMARY

Described herein are compounds such as macromolecules that have been modified in order to facilitate crosslinking. In one aspect, the macromolecule is modified via an alkoxyamination reaction, wherein the resultant alkoxyaminated macromolecule can undergo crosslinking with itself or another macromolecule. In

another aspect, the macromolecule is modified with a group capable of reacting with a hydrazide compound, which will facilitate crosslinking. Also described herein are methods of making and using the modified macromolecules.

The advantages described herein will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the aspects described below. The advantages described below will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

Figure 1 shows a reaction scheme for producing a bis(aminooxy) ether compound.

Figure 2 shows a reaction scheme for producing aminooxy ether compounds and thiolated aminooxy-modified hyaluronan.

Figure 3 shows a reaction scheme for producing aminooxy-modified hyaluronan.

Figure 4 shows a reaction scheme for producing thiolated hydrazide-modified carboxymethylhyaluronan.

Figure 5 is the ^{1}H NMR spectrum of CM-HA.

Figure 6 is the ^{1}H NMR spectrum of CM-HA-DTPH.

Figure 7 is the ^{1}H NMR spectrum of HA-aminooxy ether.

DETAILED DESCRIPTION

Before the present compounds, composites, compositions, and/or methods are disclosed and described, it is to be understood that the aspects described below

are not limited to specific compounds, synthetic methods, or uses as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings:

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not. For example, the phrase "optionally substituted lower alkyl" means that the lower alkyl group can or can not be substituted and that the description includes both unsubstituted lower alkyl and lower alkyl where there is substitution.

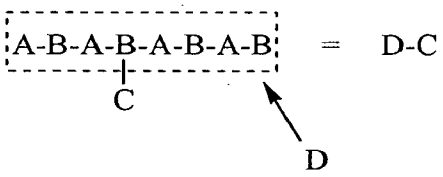
Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

References in the specification and concluding claims to parts by weight, of a particular element or component in a composition or article, denotes the weight relationship between the element or component and any other elements or components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight of component X and 5 parts by

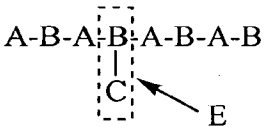
weight component Y, X and Y are present at a weight ratio of 2:5, and are present in such ratio regardless of whether additional components are contained in the compound.

A weight percent of a component, unless specifically stated to the contrary, is based on the total weight of the formulation or composition in which the component is included.

A "residue" of a chemical species, as used in the specification and concluding claims, refers to the moiety that is the resulting product of the chemical species in a particular reaction scheme or subsequent formulation or chemical product, regardless of whether the moiety is actually obtained from the chemical species. For example, a polymer having the repeat unit A-B, where one of the B units is modified with C, the resultant polymer can be represented by the formula D-C, where D is the remainder (*i.e.*, residue) of the polymer A-B.



A fragment, as used in the specification and concluding claims, refers to a portion or section of a macromolecule or the entire macromolecule. For example, a polymer having the repeat unit A-B is depicted below, where one of the B repeat units is modified with C. The B-C unit is fragment E of the polymer composed of the repeat unit A-B as depicted below.



Variables such as R^1-R^5, R^7, R^8,R^{20}, R^{25}-R^{30}, n, n', LG, A E, L, J, G, M, Q, U, V, W, X, Y, and Z used throughout the application are the same

variables as previously defined unless stated to the contrary.

The term "alkyl group" as used herein is a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, pentyl, hexyl, heptyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like. A "lower alkyl" group is an alkyl group containing from one to six carbon atoms.

The term "polyalkylene group" as used herein is a group having two or more CH_2 groups linked to one another. The polyalkylene group can be represented by the formula $-(CH_2)_n-$, where n is an integer of from 2 to 25.

The term "polyether group" as used herein is a group having the formula $-[(CHR)_nO]_m-$, where R is hydrogen or a lower alkyl group, n is an integer of from 1 to 20, and m is an integer of from 1 to 100. Examples of polyether groups include, polyethylene oxide, polypropylene oxide, and polybutylene oxide.

The term "polythioether group" as used herein is a group having the formula $-[(CHR)_nS]_m-$, where R is hydrogen or a lower alkyl group, n is an integer of from 1 to 20, and m is an integer of from 1 to 100.

The term "polyimino group" as used herein is a group having the formula $-[(CHR)_nNR]_m-$, where each R is, independently, hydrogen or a lower alkyl group, n is an integer of from 1 to 20, and m is an integer of from 1 to 100.

The term "polyester group" as used herein is a group that is produced by the reaction between a compound having at least two carboxylic acid groups with a compound having at least two hydroxyl groups.

The term "polyamide group" as used herein is a group that is produced by the reaction between a compound having at least two carboxylic acid groups with a compound having at least two unsubstituted or monosubstituted amino groups.

The term "aryl group" as used herein is any carbon-based aromatic group including, but not limited to, benzene, naphthalene, etc. The term "aromatic" also

includes "heteroaryl group," which is defined as an aromatic group that has at least one heteroatom incorporated within the ring of the aromatic group. Examples of heteroatoms include, but are not limited to, nitrogen, oxygen, sulfur, and phosphorus. The aryl group can be substituted or unsubstituted. The aryl group can be substituted with one or more groups including, but not limited to, alkyl, alkynyl, alkenyl, aryl, halide, nitro, amino, ester, ketone, aldehyde, hydroxy, carboxylic acid, or alkoxy.

The term "hydrocarbyl group" as used herein means the monovalent moiety obtained upon removal of a hydrogen atom from a parent hydrocarbon. Representative of hydrocarbyl are alkyl of 1 to 20 carbon atoms, inclusive, such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, undecyl, decyl, dodecyl, octadecyl, nonodecyl, eicosyl, heneicosyl, docosyl, tricosyl, tetracosyl, pentacosyl and the isomeric forms thereof; aryl of 6 to 12 carbon atoms, inclusive, such as phenyl, tolyl, xylyl, naphthyl, biphenyl, tetraphenyl and the like; aralkyl of 7 to 12 carbon atoms, inclusive, such as benzyl, phenethyl, phenpropyl, phenbutyl, phenhexyl, napthoctyl and the like; cycloalkyl of 3 to 8 carbon atoms, inclusive, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and the like; alkenyl of 2 to 10 carbon atoms, inclusive, such as vinyl, allyl, butenyl, pentenyl, hexenyl, octenyl, nonenyl, decenyl, undececyl, dodecenyl, tridecenyl, pentadecenyl, octadecenyl, pentacosynyl and isomeric forms thereof. Preferably, the hydrocarbyl group has 1 to 20 carbon atoms, inclusive.

The term "substituted hydrocarbyl and heterocarbyl" as used herein means the hydrocarbyl or heterocarbyl moiety as previously defined wherein one or more hydrogen atoms have been replaced with a chemical group, which does not adversely affect the desired preparation of the modified polysaccharide. Representative of such groups are amino, phosphino, quaternary nitrogen (ammonium), quaternary phosphorous (phosphonium), hydroxyl, amide, alkoxy,

mercapto, nitro, alkyl, halo, sulfone, sulfoxide, phosphate, phosphite, carboxylate, carbamate groups and the like.

The term "hydrazide compound" as used herein is any compound having at least one hydrazide group having the formula $NH_2NRC(O)$-, wherein R can be hydrogen, a lower alkyl group, an amide group, a carbamate group, a hydroxyl group, or a halogen group.

The term "hydrazide-reactive group" as used herein is any group that can react with the primary or secondary amino group of the hydrazide group to form a new covalent bond. Examples of hydrazide-reactive groups include, but are not limited to, a ketone, an aldehyde, or an activated carboxylate group.

The term "aminooxy ether compound" as used herein is any compound having the formula RONHR', wherein R can be substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof and R' can be hydrogen or a lower alkyl group. The –ONHR' group is referred to herein as an aminooxy group.

The term "aminooxy-reactive group" as used herein is any group that can react with the amino group of the aminooxy group to form a new covalent bond. Examples of aminooxy-reactive groups include, but are not limited to, a ketone, an aldehyde, or an activated carboxylate group.

A. Materials

Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed method and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may

not be explicitly disclosed, each is specifically contemplated and described herein. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited, each is individually and collectively contemplated. Thus, in this example, each of the combinations A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods, and that each such combination is specifically contemplated and should be considered disclosed.

1. Macromolecules

A macromolecule as disclosed herein is any compound having at least one hydrazide-reactive group and/or aminooxy-reactive group. Examples of hydrazide-reactive groups and aminooxy-reactive groups include, but are not limited to, a carboxyl group including the salt or ester thereof or an amide group. The hydrazide-reactive group or the aminooxy-reactive group can be naturally present on the macromolecule, or the macromolecule can be chemically modified to incorporate the hydrazide-reactive group or the aminoalkoxy-reactive group on the macromolecule.

In one aspect, the macromolecule is an oligonucleotide, a nucleic acid or a metabolically stabilized analogue thereof, a polypeptide, a lipid, a glycoprotein, or a

glycolipid. In another aspect, the macromolecule is a polysaccharide, a protein, or a synthetic polymer.

a) Oligonucleotides

The term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent intersugar (backbone) linkages as well as modified oligonucleotides having non-naturally-occurring portions which function similarly. An oligonucleotide is a polymer of repeating units generically known as nucleotides or nucleosides. An unmodified (naturally occurring) nucleotide has three components: (1) a nitrogenous base linked by one of its nitrogen atoms to (2) a 5-carbon cyclic sugar and (3) a phosphate, esterified to carbon 5 of the sugar. When incorporated into an oligonucleotide chain, the phosphate of a first nucleotide is also esterified to carbon 3 of the sugar of a second, adjacent nucleotide. The "backbone" of an unmodified oligonucleotide consists of (2) and (3), that is, sugars linked together by phosphodiester linkages between the CS (5') position of the sugar of a first nucleotide and the C3 (3') position of a second, adjacent nucleotide. Oligonucleotides can be composed of nucleoside or nucleotide sequences sufficient in identity and number to effect specific hybridization with a particular nucleic acid.

(1) Nucleic acids

Nucleic acids such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and peptide nucleic acid (PNA) are polymeric, polyionic molecules soluble in aqueous solution under certain conditions. The assumed three-dimensional structures of nucleic acids in solution as a function of pH, ionic strength, counter ions, charge neutralization, hydration, organic precipitants, molecular composition, etc., are known by those skilled in the art. In one aspect, the nucleic acid can be single or double stranded DNA or RNA.

There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids as well as any other proteins disclosed herein, as well as various functional nucleic acids. The disclosed nucleic acids are made up of for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector is expressed in a cell, the expressed mRNA will typically be made up of A, C, G, and U.

(2) Nucleotides and related molecules

A nucleotide is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), and thymin-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. An non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

A nucleotide analog is a nucleotide which contains some type of modification to either the base, sugar, or phosphate moieties. Modifications to nucleotides are well known in the art and would include for example, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, and 2-aminoadenine as well as modifications at the sugar or phosphate moieties.

Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety. (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556),

A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH2 or O) at the C6 position of purine nucleotides.

Thus, nucleic acids are polymers made up of nucleotides, called bases generically. The nucleic acid molecules can be characterized by the number of bases that make up the nucleic acid. For example, in certain embodiments the nucleic acid analytes are at least 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 295, 300, 320, 340, 360, 380, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3200, 3400, 3600, 3800, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10000, 11000, 12000, 13000, 14000, 15000, 16000, 17000, 18000, 19000, 20000, 25000, 50000, 100000, 200000,

300000, 400000, 500000, and 1000000 bases or base pairs long. In another aspect, the DNA or RNA has at least about 1,500 bases or base pairs.

b) Pharmaceutically-acceptable Compound

In one aspect, the macromolecule can be a pharmaceutically-acceptable compound. Any of the biologically active compounds disclosed in U.S. Patent No. 6,562,363 B1, which is incorporated by reference in its entirety, can be used as a pharmaceutically-acceptable compound. In one aspect, the pharmaceutically-acceptable compound includes substances capable of preventing an infection systemically in the biological system or locally at the defect site, as for example, anti-inflammatory agents such as, but not limited to, pilocarpine, hydrocortisone, prednisolone, cortisone, diclofenac sodium, indomethacin, 6∝-methyl-prednisolone, corticosterone, dexamethasone, prednisone, and the like; antibacterial agents including, but not limited to, penicillin, cephalosporins, bacitracin, tetracycline, doxycycline, gentamycin, chloroquine, vidarabine, and the like; analgesic agents including, but not limited to, salicylic acid, acetaminophen, ibuprofen, naproxen, piroxicam, flurbiprofen, morphine, and the like; local anesthetics including, but not limited to, cocaine, lidocaine, benzocaine, and the like; immunogens (vaccines) for stimulating antibodies against hepatitis, influenza, measles, rubella, tetanus, polio, rabies, and the like; peptides including, but not limited to, leuprolide acetate (an LH-RH agonist), nafarelin, and the like. All compounds are available from Sigma Chemical Co. (Milwaukee, WI).

In another aspect, the pharmaceutically-acceptable compound can be a substance or metabolic precursor which is capable of promoting growth and survival of cells and tissues or augmenting the functioning of cells is useful, as for example, a nerve growth promoting substance such as a ganglioside, a nerve growth factor, and the like; a hard or soft tissue growth promoting agent such as fibronectin (FN), human growth hormone (HGH), a colony stimulating factor, bone morphogenic protein, platelet-derived growth factor (PDGF), insulin-derived growth factor (IGF-

I, IGF-II), transforming growth factor-alpha (TGF-alpha), transforming growth factor-beta (TGF-beta), epidermal growth factor (EGF), fibroblast growth factor (FGF), interleukin-1 (IL-1), vascular endothelial growth factor (VEGF) and keratinocyte growth factor (KGF), dried bone material, and the like; and antineoplastic agents such as methotrexate, 5-fluorouracil, adriamycin, vinblastine, cisplatin, tumor-specific antibodies conjugated to toxins, tumor necrosis factor, and the like.

In another aspect, the pharmaceutically-acceptable compound can include hormones such as progesterone, testosterone, and follicle stimulating hormone (FSH) (birth control, fertility-enhancement), insulin, and the like; antihistamines such as diphenhydramine, and the like; cardiovascular agents such as papaverine, streptokinase and the like; anti-ulcer agents such as isopropamide iodide, and the like; bronchodilators such as metaproternal sulfate, aminophylline, and the like; vasodilators such as theophylline, niacin, minoxidil, and the like; central nervous system agents such as tranquilizer, B-adrenergic blocking agent, dopamine, and the like; antipsychotic agents such as risperidone, narcotic antagonists such as naltrexone, naloxone, buprenorphine; and other like substances. All compounds are available from Sigma Chemical Co. (Milwaukee, WI).

c) Lipids

In one aspect, neutral lipids can include, but are not limited to, synthetic or natural phospholipids. Typically, though not required, a neutral lipid has two hydrocarbon chains, *e.g.*, acyl chains, and either a polar, nonpolar, or zwitterionic head group. The two hydrocarbon chains can be any length. In one aspect, the hydrocarbon chain is between about 14 to about 22 carbon atoms in length, and can have varying degrees of unsaturation. In another aspect, the neutral lipid has a high molecular weight and high melting temperature.

Neutral lipids that can be used in the methods and compositions described herein to create neutral liposomes include, but are not limited to,

phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SPM), distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), diarachidonoylphosphatidylcholine (DAPC), egg phosphatidylcholine, hydrogenated soy phosphatidylcholine (HSPC), glycosphingolipids and glycoglycerolipids, and sterols such as cholesterol, either alone or in combination with other lipids. In one aspect, the neutral lipid is distearoyl-phosphatidylcholine. Such neutral lipids can be obtained commercially or can be prepared by methods known to one of ordinary skill in the art.

Suitable anionic lipids include, but are not limited to, phospholipids that contain phosphatidylglycerol, phosphatidylserine or phosphatidic acid headgroups and two saturated fatty acid chains containing from about 14 to about 22 carbon atoms. Other suitable anionic lipids include, but are not limited to, phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidic acid (PA), phosphatidylinositol (PI), cardiolipin, dimyristoylphosphatidylglycerol (DMPG), and dipalmitoylphosphatidylglycerol (DPPG). In one aspect, the anionic lipid is dimyristoylphosphatidylglycerol. Such anionic lipids can be obtained commercially or can be prepared by methods known to one of ordinary skill in the art.

In one aspect, the lipid can be any phosphoinositide in which the inositol head group has zero, one, or two phosphates. In another aspect, the lipid can be a lysolipid including, but not limited to, lysophosphatidic acid (LPA), lysophosphatidylcholines (LPCs), and lysophosphatidylinositol (LPI). In another aspect, the lipid can be a sphingolipid including, but not limited to, sphingosine-1-phosphate (S1P) or sphingophatidylcholines (LPC). In another aspect, the lipis can be ceramide.

d) Polysaccharides

Any polysaccharide known in the art can be used herein. Examples of polysaccharides include starch, cellulose, glycogen or carboxylated polysaccharides such as alginic acid, pectin, or carboxymethylcellulose. In one aspect, the

polysaccharide is a glycosaminoglycan (GAG). A GAG is one molecule with many alternating subunits. For example, HA is (GlcNAc-GlcUA-)x. Other GAGs are sulfated at different sugars. Generically, GAGs are represented by the formula A-B-A-B-A-B, where A is a uronic acid and B is an aminosugar that is either O- or N-sulfated, where the A and B units can be heterogeneous with respect to epimeric content or sulfation.

There are many different types of GAGs, having commonly understood structures, which, for example, are within the disclosed compositions, such as chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, or heparan sulfate. Any GAG known in the art can be used in any of the methods described herein. Glycosaminoglycans can be purchased from Sigma, and many other biochemical suppliers. Alginic acid, pectin, and carboxymethylcellulose are among other carboxylic acid containing polysaccharides useful in the methods described herein.

In one aspect, the polysaccharide is hyaluronan (HA). HA is a non-sulfated GAG. Hyaluronan is a well known, naturally occurring, water soluble polysaccharide composed of two alternatively linked sugars, D-glucuronic acid and N-acetylglucosamine. The polymer is hydrophilic and highly viscous in aqueous solution at relatively low solute concentrations. It often occurs naturally as the sodium salt, sodium hyaluronate. Methods of preparing commercially available hyaluronan and salts thereof are well known. Hyaluronan can be purchased from Seikagaku Company, Clear Solutions Biotech, Inc., Pharmacia Inc., Sigma Inc., and many other suppliers. For high molecular weight hyaluronan it is often in the range of 100 to 10,000 disaccharide units. In another aspect, the lower limit of the molecular weight of the hyaluronan is from 1,000 Da, 2,000 Da, 3,000 Da, 4,000 Da, 5,000 Da, 6,000 Da, 7,000 Da, 8,000 Da, 9,000 Da, 10,000 Da, 20,000 Da, 30,000 Da, 40,000 Da, 50,000 Da, 60,000 Da, 70,000 Da, 80,000 Da, 90,000 Da, or 100,000 Da, and the upper limit is 200,000 Da, 300,000 Da, 400,000 Da, 500,000

Da, 600,000 Da, 700,000 Da, 800,000 Da, 900,000 Da, 1,000,000 Da, 2,000,000 Da, 4,000,000 Da, 6,000,000 Da, 8,000,000 Da, or 10,000,000 Da where any of the lower limits can be combined with any of the upper limits. In another aspect, Y in formula III is not hyaluronan.

(1) Modified-glycosaminoglycans

In one aspect, any glycosaminoglycan in the art can be chemically modified so that at least one of the hydroxyl groups present on the glycosaminoglycan is substituted with a hydrazide-reactive group to produce a modified-glycosaminoglycan. Glycosaminoglycans in general possess a plurality of hydroxyl groups. The phrase "at least one of the hydroxyl groups present on the glycosaminoglycan is chemically substituted with a hydrazide-reactive group or aminooxy-reactive group" as used herein refers to replacing or substituting hydrogen of the hydroxyl group with the hydrazide-reactive group or the aminooxy-reactive group via a chemical manipulation of the hydroxyl group present on the glycosaminoglycan.

In one aspect, the modified-glycosaminoglycan is produced by (a) reacting a glycosaminoglycan with a base to produce deprotonated-glycosaminoglycan, and (b) reacting the deprotonated-glycosaminoglycan with a compound comprising at least one hydrazide-reactive group or aminooxy-reactive group. Not wishing to be bound by theory, it is believed that the base deprotonates at least one hydroxyl group to produce the corresponding alkoxide of the glycosaminoglycan. The alkoxide, which may be transient in nature, then reacts with the compound having at least one hydrazide-reactive group or aminooxy-reactive group to produce the modified-glycosaminoglycan. The deprotonated glycosaminoglycan may or may not react with the hydrazide-reactive group or the aminooxy-reactive group depending upon reaction conditions. Steps (a) and (b) can be performed stepwise, where the deprotonated glycosaminoglycan is isolated after step (a) followed by step (b) or, alternatively, steps (a) and (b) can be performed sequentially *in situ*.

Depending upon reaction conditions such as pH, reaction temperature, solvent, and base, any of the hydroxyl groups present on the glycosaminoglycan can be substituted with the hydrazide-reactive group or the aminooxy-reactive group. Additionally, the number of hydroxyl groups that are substituted with the hydrazide-reactive group or the aminooxy-reactive group will vary depending upon the reaction conditions. The reaction conditions for carrying out the synthesis of the modified-glycosaminoglycan are discussed below.

Any base known in the art can be used to produce the deprotonated glycosaminoglycan. Examples of bases useful herein include, but are not limited to, the base comprises a hydroxide, an alkoxide, a carbonate, an amine, phosphate, or an amide. In one aspect, sodium, potassium, or ammonium hydroxides, alkoxides, and carbonates can be used. Examples of amides useful in the present invention include, but are not limited to, potassium hexamethyldisilazide, sodium hexamethyldisilazide, lithium diisopropylamide, lithium hexamethyldisilazide, and lithium 2,2,6,6-tetramethylpiperidide. It is understood to one of ordinary skill in the art that non-aqueous solvents should be employed when the base is an amide. Examples of secondary amines include, but are not limited to, morpholine, diisopropylamine, pyrrolidine, 2,2,6,6-tetramethylpiperidine. Examples of tertiary amines include, but are not limited to, dimethyl ethyl amine, triethylamine, pyridine, diisopropylethylamine, collidine, or diazabicyclononane (DABCO).

The amount of base used to deprotonate the glycosaminoglycan will also vary depending upon the desired degree of substitution. In one aspect, when deprotonation is performed in an aqueous solution, an excess of base relative to the glycosaminoglycan is used in order to ensure sufficient deprotonation.

The synthesis of the modified-glycosaminoglycan is generally conducted in the presence of a solvent. Water, an organic solvent, or a combination thereof can be used as the reaction solvent. In one aspect, the organic solvent can be an alcohol, an ether, or a halogenated solvent. Examples of organic solvents useful in the

present invention include, but are not limited to, dichloromethane, dimethylformamide, dimethylsulfoxide, dioxane, N-methylmorpholine, sulfolane, N-methylpyrrolidone, tetrahydrofuran, diethyl ether, toluene, dimethoxyethane, t-butyl methyl ether, or a mixture thereof.

Reaction temperatures and times can vary when adding the base to the glycosaminoglycan. In one aspect, the base is added to the glycosaminoglycan from -50 °C to 80 °C. In another aspect, the lower limit of the reaction temperature is -45 °C, -40 °C, -35 °C, -30 °C, -25 °C, -20 °C, or -15 °C, and the upper limit is -5 °C, -10 °C, -15 °C, -20 °C, -25 °C, 0 °C, 20 °C, 40 °C, or 60°C, where any lower temperature limit can be combined with any upper temperature limit. The base is allowed to react with glycosaminoglycan at from 30 seconds to 100 hours. In another, the lower time limit can be 1, 5, 10, 15 minutes, and the upper limit can be 100 hours, 90 hours, 80 hours, 70 hours, 60 hours, 50 hours, 40 hours, 30 hours, 20 hours, 10 hours, 5 hours, 2 hours, 1 hour, 30 minutes, 15 minutes, 10 minutes, or 5 minutes, where any lower time limit can be combined with any upper time limit.

After the deprotonated glycosaminoglycan is produced, a compound having at least one hydrazide-reactive group or aminooxy-reactive group is allowed to react with the deprotonated glycosaminoglycan. Any compound that possesses a hydrazide-reactive group and/or an aminooxy-reactive group that is capable of reacting with the deprotonated glycosaminoglycan can be used to produce the modified-glycosaminoglycan. In one aspect, the compound having at least one hydrazide-reactive group and/or an aminooxy-reactive group possesses a leaving group, wherein upon reaction with the deprotonated glycosaminoglycan, the bond between the leaving group and the compound is broken and a new bond is formed between the oxygen of the deprotonated glycosaminoglycan and the atom that was bonded to the leaving group, which is usually carbon. A leaving group is any group that is readily liberated from a compound when that compound is allowed to react with a nucleophile. Examples of leaving groups include, but are not limited to, a

halogen such as fluoro, chloro, bromo, or iodo, a carbonate, ammonium group, or activated leaving groups such as tosylate, mesylate, phosphate, or triflate. The use of leaving groups for forming new bonds by nucleophilic substitution is widely known in the art. In another aspect, when the hydrazide-reactive group or the aminooxy-reactive group is an ester, the ester is can be activated with a leaving group including, but not limited to, an ammonium group, or a tosylate, mesylate, phosphate, or triflate, where the leaving group is bonded to the carbonyl carbon.

In one aspect, the compound having at least one hydrazide-reactive group or aminooxy group has the formula LG-L-G, wherein LG is a leaving group; L is a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof; and G is a hydrazide-reactive group or aminooxy-reactive group as defined above. In one aspect, LG can be a halogen. In another aspect, L can be a polyalkylene group having the formula $(CH_2)_n$, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In another aspect, G can be CO_2H or the salt or ester thereof. When the compound having the formula LG-L-G reacts with the deprotonated glycosaminoglycan, a covalent bond is formed between the deprotonated oxygen of the hydroxyl group and L and LG^- is produced.

As described above, the selectivity and degree of substitution of the glycosaminoglycan will vary depending upon the reaction conditions selected. For example, depending upon the glycosaminoglycan selected, certain hydroxyl protons are more acidic than others. Thus by varying the pH (*i.e.*, the amount and type of base) in the deprotonation step, it is possible to preferentially deprotonate one class of hydroxyl groups over another. In one aspect, a single hydroxyl group to 100 % of the hydroxyl groups present on the glycosaminoglycan can be deprotonated and substituted.

In one aspect, the primary hydroxyl group of the glycosaminoglycan is chemically substituted with a hydrazide-reactive group or an aminooxy-reactive group. When the glycosaminoglycan is any compound other than hyaluronan, the primary hydroxyl group of the glycosaminoglycan is the C-6 hydroxyl group of the non-uronic acid sugar component of the repeating disaccharide of the glycosaminoglycan. All other hydroxyl groups present in the glycosaminoglycan are referred to herein as secondary hydroxyl groups.

In one aspect, when the glycosaminoglycan is hyaluronan, at least one primary hydroxyl group is chemically substituted with the hydrazide-reactive group or the aminooxy-reactive group. In the case of hyaluronan, the primary hydroxyl group is the C-6 hydroxyl group of the N-acetyl-glucosamine residue. All other hydroxyl groups present in hyaluronan that are not the primary hydroxyl group are referred to herein as the secondary hydroxyl group.

In one aspect, one primary hydroxyl group of the glycosaminoglycan to 100 % of the primary hydroxyl groups can be substituted with the hydrazide-reactive group or aminooxy-reactive group. In one aspect, 0.1% to 40%, 0.1% to 30%, 0.1% to 20%, 0.1% to 10%, or 0.1% to 5% of the primary hydroxyl groups of hyaluronan can be substituted. In another aspect, 0.1%, 0.5%, 1%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, or 30% of the primary hydroxyl groups to 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the primary hydroxyl groups of hyaluronan can be substituted, where any lower endpoint can be combined with any upper endpoint. In another aspect, when one or more primary hydroxyl groups of the glycosaminoglycan are substituted, one or more secondary hydroxyl groups can also be substituted with the hydrazide-reactive group or the aminooxy-reactive group depending upon reaction conditions.

In one aspect, the modified-glycosaminoglycan can be hyaluronan with at least one primary hydroxyl group substituted with CH_2CO_2H or the salt or ester

thereof, wherein the CH_2 group is covalently bonded to oxide of the deprotonated glycosaminoglycan.

(2) Glycolipids and Glycoproteins

In one aspect, the macromolecule can be a glycolipid having at least one hydrazide-reactive group or aminooxy-reactive group. Examples of glycolipids include, but are not limited to, MGDG, diacylglucopyranosyl glycerols, and Lipid A. The glycolipids disclosed in U.S. patent no. 6,635,622, which is incorporated by reference in its entirety, can be used herein.

In another aspect, the macromolecule can be a glycoprotein having at least one hydrazide-reactive group or aminooxy-reactive group. Examples of glycolipids include, but are not limited to, orosomucoid alpha-1-acid glycoprotein (AAG) and alpha-1-glycoprotein. The glycolipids disclosed in U.S. patent no. 6,617,450 and 6,656,714, which are incorporated by reference in their entirety, can be used herein.

e) Synthetic polymers

Any synthetic polymer known in the art can be used in the compositions and methods described herein. In one aspect, the synthetic polymer is glucuronic acid, polyacrylic acid, polyaspartic acid, polytartaric acid, polyglutamic acid, or polyfumaric acid.

f) Proteins

Any type of protein can be used in the compositions and methods described herein. For example, the protein can include peptides or fragments of proteins or peptides. The protein can be of any length, and can include one or more amino acids or variants thereof. The protein(s) can be fragmented, such as by protease digestion, prior to analysis.

Proteins useful in the methods described herein include, but are not limited to, an extracellular matrix protein, a chemically-modified extracellular matrix protein, or a partially hydrolyzed derivative of an extracellular matrix protein. The

proteins may be naturally occurring or recombinant polypeptides possessing a cell interactive domain. The protein can also be mixtures of proteins, where one or more of the proteins are modified. Specific examples of proteins include, but are not limited to, collagen, elastin, decorin, laminin, or fibronectin.

(1) Protein variants

As discussed herein there are numerous variants of proteins and that are known and herein contemplated. Protein variants and derivatives are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions

preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Tables 1 and 2 and are referred to as conservative substitutions.

TABLE 1:Amino Acid Abbreviations

Amino Acid	Abbreviations
alanine	Ala (A)
alloisoleucine	AIle
arginine	Arg (R)
asparagine	Asn (N)
aspartic acid	Asp (D)
cysteine	Cys (C)
glutamic acid	Glu (E)
glutamine	Gln (K)
glycine	Gly (G)
histidine	His (H)
isolelucine	Ile (I)
leucine	Leu (L)
lysine	Lys (K)
phenylalanine	Phe (F)

Amino Acid	Abbreviations
proline	Pro (P)
pyroglutamic acid	Glu
serine	Ser (S)
threonine	Thr (T)
tyrosine	Tyr(Y)
tryptophan	Trp (W)
valine	Val (V)

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TABLE 1:Amino Acid Substitutions
Original Residue Exemplary Conservative Substitutions, others are known in the art.
Ala ↔ ser
Arg ↔ lys or gln
Asn ↔ gln or his
Asp ↔ glu
Cys ↔ ser
Gln ↔ asn or lys
Glu ↔ asp
Gly ↔ pro
His ↔ asn or gln
Ile ↔ leu or val
Leu ↔ ile or val
Lys ↔ arg or gln;
Met ↔ Leu or ile
Phemet ↔ leu or tyr
Ser ↔ thr
Thr ↔ ser
Trp ↔ tyr
Tyr ↔ trp or phe
Val ↔ ile or leu

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.*

183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70% homology to a particular sequence wherein the variants are conservative mutations.

As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism, where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein from which that protein arises is also known and herein disclosed and described.

It is understood that there are numerous amino acid and peptide analogs which can be incorporated into the disclosed compositions. For example, there are numerous D amino acids or amino acids which have a different functional substituent then the amino acids shown in Table 1 and Table 2. The opposite stereo isomers of naturally occurring peptides are disclosed, as well as the stereo isomers of peptide analogs. These amino acids can readily be incorporated into polypeptide chains by charging tRNA molecules with the amino acid of choice and engineering genetic constructs that utilize, for example, amber codons, to insert the analog amino acid into a peptide chain in a site specific way (Thorson et al., Methods in Molec.

Biol. 77:43-73 (1991), Zoller, Current Opinion in Biotechnology, 3:348-354 (1992); Ibba, Biotechnology & Genetic Enginerring Reviews 13:197-216 (1995), Cahill et al., TIBS, 14(10):400-403 (1989); Benner, TIB Tech, 12:158-163 (1994); Ibba and Hennecke, Bio/technology, 12:678-682 (1994) all of which are herein incorporated by reference at least for material related to amino acid analogs).

Molecules can be produced that resemble peptides, but which are not connected via a natural peptide linkage. For example, linkages for amino acids or amino acid analogs can include CH_2NH--, --CH_2S--, --CH_2--CH_2 --, --CH=CH-- (cis and trans), --$COCH_2$ --, --$CH(OH)CH_2$--, and --CHH_2SO—(These and others can be found in Spatola, A. F. in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Spatola, A. F., Vega Data (March 1983), Vol. 1, Issue 3, Peptide Backbone Modifications (general review); Morley, Trends Pharm Sci (1980) pp. 463-468; Hudson, D. et al., Int J Pept Prot Res 14:177-185 (1979) (--CH_2NH--, CH_2CH_2--); Spatola et al. Life Sci 38:1243-1249 (1986) (--CH H_2--S); Hann J. Chem. Soc Perkin Trans. I 307-314 (1982) (--CH--CH--, cis and trans); Almquist et al. J. Med. Chem. 23:1392-1398 (1980) (--$COCH_2$--); Jennings-White et al. Tetrahedron Lett 23:2533 (1982) (--$COCH_2$--); Szelke et al. European Appln, EP 45665 CA (1982): 97:39405 (1982) (--$CH(OH)CH_2$--); Holladay et al. Tetrahedron. Lett 24:4401-4404 (1983) (--$C(OH)CH_2$--); and Hruby Life Sci 31:189-199 (1982) (--CH_2--S--); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is --CH_2NH--. It is understood that peptide analogs can have more than one atom between the bond atoms, such as b-alanine, g-aminobutyric acid, and the like.

Amino acid analogs and analogs and peptide analogs often have enhanced or desirable properties, such as, more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others.

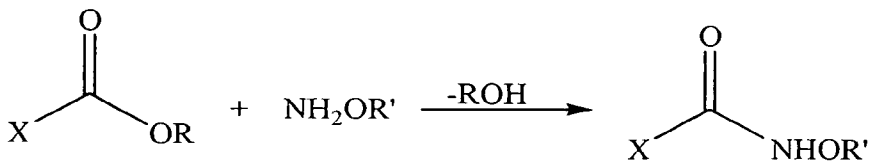
D-amino acids can be used to generate more stable peptides, because D amino acids are not recognized by peptidases and such. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) can be used to generate more stable peptides. Cysteine residues can be used to cyclize or attach two or more peptides together. This can be beneficial to constrain peptides into particular conformations. (Rizo and Gierasch Ann. Rev. Biochem. 61:387 (1992), incorporated herein by reference).

2. Modification of Macromolecules

Described below are modifications of macromolecules using the methods and compositions described herein. The modifications generally involve the alkoxyamination of a macromolecule to produce an aminooxy-modified macromolecule, the hydrazide-modification of a macromolecule to produce a hydrazide-modified macromolecule, or a combination thereof. Any of the macromolecules described above, including the modified glycosaminoglycans, can be modified using the methods and compositions described below.

a) Alkoxyamination

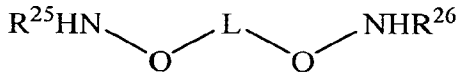
Alkoxyamination involves reacting any of the macromolecules described above with a compound having at least one aminooxy group. A general reaction scheme that shows the reaction between a carboxylic acid group of macromolecule X, which is an aminooxy-reactive group, and an aminooxy ether compound is depicted in Scheme 1.



SCHEME 1

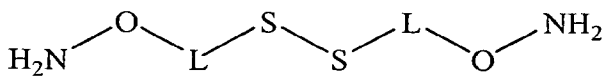
The aminooxy group can react with any aminooxy-reactive group present on the macromolecule. Thus, in one aspect, the aminooxy group can react with a naturally-occurring aminooxy-reactive group present on the macromolecule. For example, hyaluronan has a plurality of COOH groups that can behave as aminooxy-reactive groups. In another aspect, when the macromolecule is any of the modified-glycosaminoglycans described above, the aminooxy group can react with the naturally-occurring aminooxy-reactive group present on the modified-glycosaminoglycan and/or the new aminooxy-reactive group that was chemically incorporated into the glycosaminoglycan. For example, in Figure 3, compound **F** can be reacted with $RONH_2$ to produce compound **I**, where the aminooxy ether compound reacted with naturally-occurring COOH group of the glucuronic acid unit, and compound **J**, where the aminooxy ether compound reacted with the C-6 carboxymethyl group of the N-acetyl-glucosamine unit.

In one aspect, the aminooxy ether compound has the formula XXV



XXV

where L can be a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof, and R^{25} and R^{26} can be, independently, hydrogen, alkyl, or aryl. In one aspect, L can be a polyalkylene having a disulfide linkage (-S-S-). In another aspect, the aminooxy ether compound has the formula XXVI



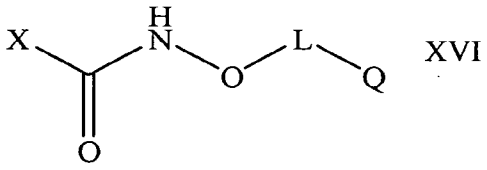
XXVI

where each L can be, independently, a polyalkylene group or an aryl group. Reaction schemes for making an aminooxy ether compound useful in the methods and compositions described herein are depicted in Figures 1 and 2.

In one aspect, the reaction between the aminooxy ether compound and the macromolecule is carried out under mild conditions at a pH of about 0 to about 8, about 1 to about 7, or about 2 to about 6, or about 3 to about 5. In one aspect, the macromolecule is dissolved in water, which may also contain water-miscible solvents including, but not limited to, dimethylformamide, dimethylsulfoxide, and hydrocarbyl alcohols, diols, or glycerols.

The number of aminooxy groups present on the aminooxy-modified macromolecule will vary depending upon the amounts of aminooxy ether compound and macromolecule used. In one aspect, 1% to 99%, 10% to 90%, 20% to 80%, 30% to 70%, or 40% to 50% of the aminooxy-reactive groups present on the macromolecule are converted to the aminooxy group. In one aspect, at least one molar equivalent of aminooxy ether compound per molar equivalent of macromolecule is added. In other aspects, for maximum percentage functionalization, a large molar excess of the aminooxy ether compound (*e.g.*, 10-100 fold) dissolved in water or aqueous-organic mixture is added and the pH of the reaction mixture is adjusted by the addition of dilute acid, *e.g.*, HCl. In one aspect, a condensing agent can be used to facilitate the reaction between the macromolecule and the aminooxy ether compound. Examples of condensing agent useful herein include, but are not limited to, a water soluble carbodiimide such as 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDCI). In another aspect, the condensing agent can be a hydroxybenzotriazole. In another aspect, an active ester forming agent such as N-hydroxysulfosuccinimide (sulfo-NHS) or N-hydroysuccinimide (NHS) can be used in combination with the condensing agent. The active ester forming agents disclosed in U.S. patent no. 6,630,457, which is incorporated by reference in its entirety, can be used herein. A sufficient molar excess (*e.g.*, 2 to 100 fold) of carbodiimide reagent dissolved in water, in any aqueous-organic mixture, or finely-divided in solid form is then added to the reaction mixture.

In one aspect, after the macromolecule has reacted with the aminooxy ether compound, the resultant modified macromolecule has at least one fragment having the formula XVI



XVI

wherein

X can be a residue of macromolecule;

Q can be a bioactive agent, an aminooxy group, a SH group, or a thiol-reactive electrophilic functional group; and

L can be a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

In formula XVI, X can be a residue of any of the macromolecules described herein. In one aspect, X is a residue of a modified-glycosaminoglycan described herein. In another aspect, L can be a polyalkylene group having the formula $(CH_2)_n$, wherein n is from 1 to 10, 1 to 8, 1 to 6, 1 to 4, 1 to 3, or 2.

In one aspect, Q in formula XVI can be a bioactive agent. The term "bioactive agent" as used herein is any therapeutic, prophylactic, pharmacological or physiological active substance, or mixture thereof, which is delivered to a subject to produce a desired, usually beneficial, effect. In one aspect, any active agent that is capable of producing a pharmacological response, localized or systemic, irrespective of whether therapeutic, diagnostic or prophylactic in nature, can be used as bioactive agents in any of the methods and compositions described herein. It should be noted that the bioactive agent can be used singularly or as a mixture of two or more agents.

Thus, it is possible to have two or more bioactive agents covalently attached to the macromolecule via the aminooxy ether compound. In one aspect, any of the macromolecules described above can be used as the bioactive agent. In another aspect, the bioactive agent can be a dye, a probe, a nucleic acid, an enzyme, an oligonucleotide, a label, a protein, a polypeptide, a lipid, a glycoprotein, a glycolipid, or a pharmaceutically-acceptable compound. In another aspect, any of the bioactive agents disclosed in U.S. Patent No. 6,562,363 B1, which is incorporated by reference in its entirety, can be used herein.

In one aspect, the bioactive agent can be linked to the aminooxy ether compound via a linkage. Examples of linkages include, but are not limited to, ethers, imidates, thioimidates, esters, amides, thioethers, thioesters, thioamides, carbamates, ethers, disulfides, hydrazides, hydrazones, oxime ethers, oxime esters, and amines.

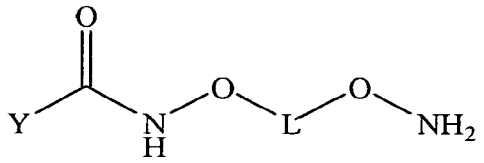
In another aspect, Q in formula XVI is a thiol-reactive electrophilic functional group. The term "thiol-reactive electrophilic group" as used herein is any group that is susceptible to nucleophilic attack by the lone-pair electrons on the sulfur atom of the thiol group or by the thiolate anion. Examples of thiol-reactive electrophilic groups include groups that have good leaving groups. For example, an alkyl group having a halide or alkoxy group attached to it or an α-halocarbonyl group are examples of thiol-reactive electrophilic groups.

In another aspect, the thiol-reactive electrophilic group is an electron-deficient vinyl group. The term "an electron-deficient vinyl group" as used herein is a group having a carbon-carbon double bond and an electron-withdrawing group attached to one of the carbon atoms. An electron-deficient vinyl group is depicted in the formula $C_\beta=C_\alpha X$, where X is the electron-withdrawing group. When the electron-withdrawing group is attached to Cα, the other carbon atom of the vinyl group (Cβ) is more susceptible to nucleophilic attack by the thiol group. This type of addition to an activated carbon-carbon double bond is referred to as a Michael

addition. In another aspect, the thiol-reactive compound can be represented by the formula C=CW, where W is the thiol-reactive electrophilic functional group. In one aspect, W can be $OC(O)R^{20}$, wherein R^{20} can be a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, or a combination thereof.

Examples of electron-withdrawing groups include, but are not limited to, a nitro group, a cyano group, an ester group, an aldehyde group, a keto group, a sulfone group, or an amide group. Examples of compounds possessing thiol-reactive electrophilic groups include, but are not limited to, maleimides, vinyl sulfones, acrylonitriles, α-methylene esters, quinone methides, acryloyl esters or amides, or α-halo esters or amides.

In another aspect, Q in formula XVI is an aminooxy group. In one aspect, a compound possessing two or more aminooxy groups, where one of the aminooxy groups does not react with an aminooxy-reactive group on the macromolecule, can result in a free aminooxy group Q. Depending upon the identity of L in formula XVI, it is possible to have two or more free or reacted aminooxy groups present in formula XVI. In one aspect, the modified macromolecule has at least one fragment having the formula II



II

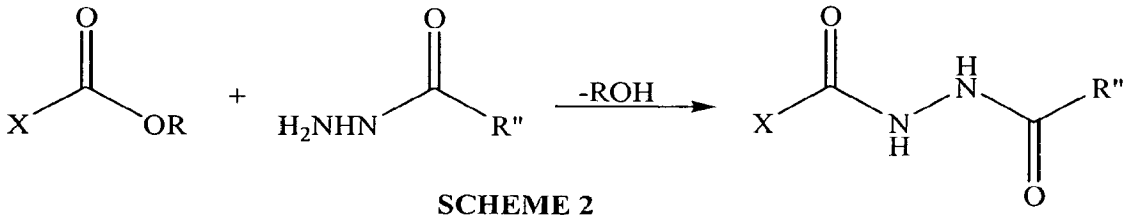
wherein Y is any modified glycosaminoglycan and linker, respectively, described herein.

In another aspect, Q in formula XVI can be SH. Figure 2 depicts one aspect of the method described above for producing a compound having the formula XVI, where Q is SH. The first step involves reacting the macromolecule hyaluronan (**A**)

having the formula HA-COOH with the aminooxy ether compound **B** to produce compound **C**. In one aspect, the reaction can be performed in the presence of a condensing agent. In one aspect, a condensing agent is any compound that facilitates the reaction between the aminooxy group of compound **B** and the COOH group on the macromolecule **A**. Any of the condensing agents described above can be used in this aspect. In one aspect, the condensing agent is a carbodiimide, including, but not limited to, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDCI). The disulfide bond in compound **C** can be cleaved with a reducing agent. In one aspect, the reducing agent is dithiothreitol. Cleavage of the disulfide bonds in compound **C** produces thiol compounds **D**, which fall under formula XVI. In one aspect, when Q is SH in formula XVI, L can be CH_2, CH_2CH_2, $CH_2CH_2CH_2$, or phenyl.

b) Hydrazide-modification

Hydrazide-modification of a macromolecule involves reacting any of the macromolecules described herein with a compound having at least one hydrazide group to produce a hydrazide-modified macromolecule. The mechanism is similar to that above in Scheme 1 for the reaction between the aminooxy ether compound and the macromolecule. Reaction scheme 2 that shows the reaction between a carboxylic acid of macromolecule X, which is a hydrazide-reactive group, and a hydrazide compound.



SCHEME 2

Similar to the aminooxy group, the hydrazide-group can react with any hydrazide-reactive group present on the macromolecule. Thus, in one aspect, the hydrazide group can react with a naturally-occurring hydrazide-reactive group

present on the macromolecule. In another aspect, when the macromolecule is any of the modified-glycosaminoglycans described above, the hydrazide group can react with the naturally-occurring hydrazide-reactive group present on the modified-glycosaminoglycan and/or the new hydrazide-reactive group that was chemically incorporated into the glycosaminoglycan.

In one aspect, a hydrazide compound can be reacted with any of the modified-glycosaminoglycans described herein to produce a hydrazide-modified macromolecule. Any of the techniques and procedures disclosed in U.S. Patent No. 5,874,417 for functionalizing hyaluronan with a hydrazide, which is incorporated by reference in its entirety, can be used to hydrazide-modify the any macromolecules described herein. For example, the modified-glycosaminoglycan can be reacted with a monohydrazide (*i.e.*, a compound having only one hydrazide group) or a polyhydrazide (*i.e.*, a compound having two or more hydrazide groups). Any of the hydrazide compounds disclosed in U.S. Patent No. 5,874,417 can be used in this aspect.

In one aspect, dihydrazides can be used to modify any of the macromolecules herein. In one aspect, the dihydrazide has the formula 1:

$$H_2N\text{--}NH\text{--}CO\text{--}A\text{--}CO\text{--}NH\text{--}NH_2 \quad (1)$$

wherein A is hydrocarbyl such as alkyl, aryl, alkylaryl or arylalkyl or A is heterohydrocarbyl, which also includes oxygen, sulfur, and/or nitrogen atoms in addition to carbon atoms. In this aspect, the alkyl group may be branched or unbranched and contain one to 20 carbons or other carbon-sized atoms, preferably 2 to 10, more preferably 4 to 8 carbons or carbon-sized heteroatoms, such as oxygen, sulfur or nitrogen. The alkyl group may be fully saturated or may contain one or more multiple bonds. The carbon atoms of the alkyl may be continuous or separated by one or more functional groups such as an oxygen atom, a keto group, an amino group, an oxycarbonyl group and the like. The alkyl group may be substituted with one or more aryl groups. The alkyl group may in whole or in part, be in form of

rings such as cyclopentyl, cyclohexyl, and the like. Any of the alkyl groups described above may have double or triple bond(s).

Any of the hydrocarbyl groups can be used as a heterocarbyl group, wherein the alkyl or aryl group contains a heteroatom such as oxygen, sulfur, or nitrogen incorporated within the chain or ring. Moreover, any of the carbon atoms of the alkyl group may be separated from each other or from the dihydrazide moiety with one or more groups such as carbonyl, oxycarbonyl, amino, and also oxygen and sulfur atoms singly or in a configuration such as --S--S--, --O--CH_2 --CH_2 --O--, S--S--CH_2 --CH_2 -- and $NH(CH_2)_nNH$--, where n is from 1 to 20.

Aryl substituents are typically substituted or unsubstituted phenyl, but may also be any other aryl group such as pyrrolyl, furanyl, thiophenyl, pyridyl, thiazoyl, etc. An inorganic, alkyl or other aryl group including halo, hydroxy, amino, thioether, oxyether, nitro, carbonyl, etc may further substitute the aryl group.

The alkylaryl or arylalkyl groups may be a combination of alkyl and aryl groups as described above. These groups may be further substituted as described above.

In another aspect, the dihydrazide has the formula (2)

$$H_2N\text{--}NH\text{--}CO\text{--}NH\text{--}A\text{--}CO\text{--}NH\text{--}NH_2 \quad (2)$$

In this aspect, A in formula 2 can be hydrocarbyl, heterocarbyl, substituted hydrocarbyl substituted heterocarbyl and the like. In another aspect, A can be any of the linkers denoted and referred to as L throughout the application.

Generally, to obtain dihydrazides, two hydroxy groups of a dicarboxylic acid are substituted with NH_2NH_2 yielding the dihydrazide. Examples of dicarboxylic acids include, but are not limited to, maleic acid, fumaric acid, and aromatic dicarboxylic acids, such as terephthalic acid and isophthalic acid.

In one embodiment, aliphatic dihydrazides, where A is an alkyl group, may have the formula 3:

$NH_2\ NHCO(CH_2)_{n'}\ CONHNH_2$ (3)

wherein n' can be any length but is preferably from 1 to 20. Aliphatic dihydrazides useful in the invention include, but are not limited to, succinic (butandioic) (n'=2), adipic (hexanedioic) (n'=4), suberic (octanedioic) (n'=6), oxalic (ethanedioic) (n'=0), malonic (propanedioic) (n'=1), glutaric (pentanedioic) (n'=3), pimelic (heptanedioic) (n'=5), azelaic (nonanedioic) (n'=7), sebacic (decanedioic) (n'=8), dodecanedioic, (n'=10), brassylic (tridecanedioic), (n'=11), (etc. up to n'=20).

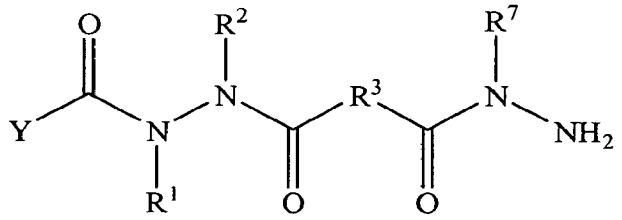
In one aspect, adipic dihydrazide, suberic dihydrazide, and butandioic dihydrazide are used to prepare the modified polysaccharide. Adipic dihydrazide can be purchased from Aldrich Chemical Co. (Milwaukee, WI). In another aspect, phthalic dihydrazide and dihydrazides with A containing oxa, thio, amino, disulfide (--CH_2 --S--S--CH_2 --), --$S(CH_2)_2$ S--, --$O(CH_2)_n$ O-- or --$NH(CH_2)_n$NH-- (n=2 to 4) groups.

In one aspect, the dihydrazides are at least partially soluble in water. The dihydrazides are also weak bases or weak acids having a pK_a for the protonated form, less than about 8, preferably in the range of 1 to 7 and most preferably 2 to 6. It will be understood that the term pK_a is used to express the extent of dissociation or the strength of weak acids, so that, for example, the pK_a of the protonated amino group of amino acids is in the range of about 12-13 in contrast to the pK_a of the protonated amino groups of the dihydrazides useful herein which is less than about 7.

As described above, the hydrazide compound reacts with a hydrazide-reactive group present on the macromolecule. In one aspect, the reaction is carried out under mild conditions at a pH of about 0 to about 8, about 1 to about 7, or about 2 to about 6, or about 3 to about 5. In one aspect, the macromolecule is dissolved in water, which may also contain water-miscible solvents including, but not limited to, dimethylformamide, dimethylsulfoxide, and hydrocarbyl alcohols, diols, or glycerols.

Similar to above for the aminooxy ether compounds, the number of hydrazide groups present on the modified macromolecule will vary depending upon the amounts of hydrazide compound and macromolecule used. In one aspect, 1% to 99%, 10% to 90%, 20% to 80%, 30% to 70%, or 40% to 50% of the hydrazide-reactive groups present on the macromolecule are converted to the hydrazide group. In one aspect, at least one molar equivalent of hydrazide compound per molar equivalent of macromolecule is added. In other aspects, for maximum percentage functionalization, a large molar excess of the hydrazide compound (*e.g.*, 10-100 fold) dissolved in water or aqueous-organic mixture is added and the pH of the reaction mixture is adjusted by the addition of dilute acid, *e.g.*, HCl. A sufficient molar excess (*e.g.*, 2 to 100 fold) of carbodiimide reagent dissolved in water, in any aqueous-organic mixture, or finely-divided in solid form is then added to the reaction mixture.

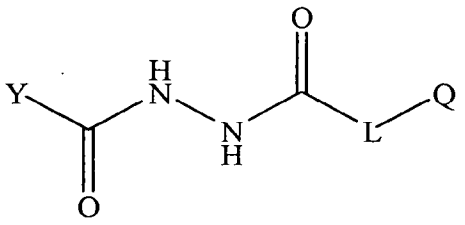
In one aspect, the hydrazide-modified macromolecule has at least one fragment having the formula I



I

wherein Y can be a residue of any modified-glycosaminoglycan described herein and R^1, R^2, R^3, and R^7 can be, independently, hydrogen, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, or a polyether group, wherein R^3 is not hydrogen. In one aspect, R^1, R^2, and $R^7$are hydrogen. In another aspect, can be R^3 can be alkyl group such as $(CH_2)_n$, wherein n is from 1 to 20, 1 to 15, 1 to 10, 1 to 8, 1 to 6, or 2 to 4.

In one aspect, the hydrazide-modified macromolecule has at least one fragment having the formula III



III

wherein Y can be a residue of any of the modified-glycosaminoglycan described herein; Q can be a residue of a bioactive agent, SH group or a thiol-reactive electrophilic functional group; and L can be a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof. Any of the bioactive agents and thiol-reactive electrophilic functional groups described above can be used in this aspect.

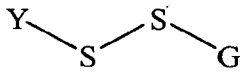
In one aspect, Q in formula III is SH. Figure 4 depicts one aspect for making compounds having the formula III where Q is SH. The modified-hyaluronan compound **F**, where a primary hydroxyl group as defined above is converted to the carboxymethyl group, is reacted with 3,3'-dithiobis(propanoic dihydrazide) (DTP) in the presence of the condensing agent such as, for example, EDCI. The hydrazide compound can react with the carboxylic acid group on the glucuronic acid unit of hyaluronan and/or the C-6 carboxymethyl group of the N-acetyl-glucosamine unit of hyaluronan. This reaction produces dihydrazide/disulfide hyaluronan that can be isolated or further manipulated *in situ*. The disulfide bond of the dihydrazide/disulfide hyaluronan can be cleaved with a reducing agent such as, for example, dithiothreitol (DTT) to produce the hydrazide/thiol compound **G** and/or **H**. In one aspect, when Q in formula III is a SH group, L can be CH_2, CH_2CH_2, or $CH_2CH_2CH_2$.

3. Crosslinked Macromolecules

Described below are methods and compositions for crosslinking any of the modified macromolecules described herein to produce a physiologically compatible macromolecular scaffold useful as a therapeutic aid. "Crosslinking" is defined herein as the ability of two or more macromolecules to produce a pore-containing matrix, where the macromolecules can be the same or different. One or more of macromolecules can be modified using any of the methods and compositions described herein. The use of additional compounds that will facilitate crosslinking are also contemplated.

a) Oxidative Coupling

In general, oxidative coupling involves reacting two or more compounds that each have a SH group in the presence of an oxidant. It is also contemplated that the thiolated compound can couple with itself as well as the other thiolated compound. The reaction between the two SH groups produces a new disulfide bond (-S-S-). In one aspect, the oxidative coupling of a first thiolated compound Y-SH and a second thiolated compound G-SH produces a compound having the fragment VII



VII

wherein Y can be a residue of any macromolecule described herein such as a modified-glycosaminoglycan and G is a residue of the thiolated compound. Depending upon the selection of the macromolecule, the macromolecule can be chemically modified so that the macromolecule has at least on SH group. For example, any naturally-occurring COOH groups or COOH groups added to the macromolecule can be converted to a thiol group using the techniques described herein including, but not limited to, the hydrazide and aminooxy methods described herein.

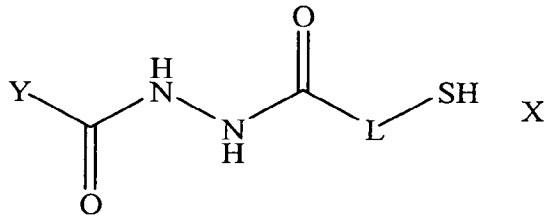
The second thiolated compound G-SH is any compound having at least one thiol group. The first and second thiolated compounds can be the same or different compounds. In one aspect, the second thiolated compound can be any macromolecule described above. In one aspect, the second thiolated compound is a polysaccharide having at least one SH group. Any of the polysaccharides described above can be used as the second thiolated compound. In another aspect, the second thiolated compound can be a sulfated-glycosaminoglycan. In a further aspect, the second thiolated compound includes chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, heparan sulfate, alginic acid, pectin, or carboxymethylcellulose, or hyaluronan, wherein each of these compounds has at least one SH group.

The reaction between the first and second thiolated compounds is performed in the presence of an oxidant. In one aspect, the reaction between the first and second thiolated compounds can be conducted in the presence of any gas that contains oxygen. In one aspect, the oxidant is air. This aspect also contemplates the addition of a second oxidant to expedite the reaction. In another aspect, the reaction can be performed under an inert atmosphere (*i.e.*, oxygen free), and an oxidant is added to the reaction. Examples of oxidants useful in this method include, but are not limited to, molecular iodine, hydrogen peroxide, alkyl hydroperoxides, peroxy acids, dialkyl sulfoxides, high valent metals such as Co^{+3} and Ce^{+4}, metal oxides of manganese, lead, and chromium, and halogen transfer agents. The oxidants disclosed in Capozzi, G.; Modena, G. In *The Chemistry of the Thiol Group Part II*; Patai, S., Ed.; Wiley: New York, 1974; pp 785-839, which is incorporated by reference in its entirety, are useful in the methods described herein.

The reaction between the first and second thiolated compounds can be conducted in a buffer solution that is slightly basic. The amount of the first thiolated compound relative the amount of the second thiolated compound can vary. In one aspect, the volume ratio of the first thiolated compound to the second thiolated

compound is from 99:1, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, or 1:99. In one aspect, the first and second thiolated compound react in air and are allowed to dry at room temperature. In this aspect, the dried material can be exposed to a second oxidant, such as hydrogen peroxide. The resultant compound can then be rinsed with water to remove any unreacted first and/or second thiolated compound and any unused oxidant. One advantage of preparing coupled compound via the oxidative coupling methodology described herein is that crosslinking can occur in an aqueous media under physiologically benign conditions without the necessity of additional crosslinking reagents.

In one aspect, described herein is a method for coupling two or more thiolated compounds by reacting a first thiolated compound having the formula X

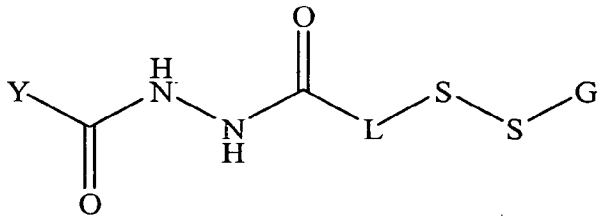


X

wherein Y can be a residue of any modified-glycosaminoglycan described herein, and L can be a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof,

with a second thiolated compound having at least one SH group in the presence of an oxidant, wherein the first thiolated compound and second thiolated compound are the same or different compounds. In one aspect, the second thiolated compound has the formula X. In a further aspect, the first and second thiolated compounds are the same compound.

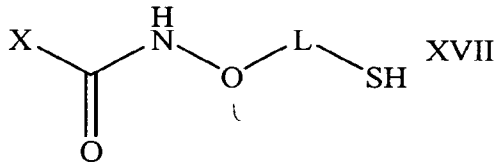
The reaction between the thiolated compound having the formula X and the second thiolated compound produces a crosslinked compound having the fragment VIII



VIII

where Y and L are as defined above. In one aspect, L in formula VIII can be CH_2, CH_2CH_2, or $CH_2CH_2CH_2$. In another aspect, G can be a polysaccharide residue such as, for example, a sulfated-glycosaminoglycan residue. In another aspect, G can be a residue of chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, heparan sulfate, alginic acid, pectin, or carboxymethylcellulose, or hyaluronan.

In another aspect, described herein is a method for coupling two or more thiolated compounds by reacting a first thiolated compound having the formula XVII



XVII

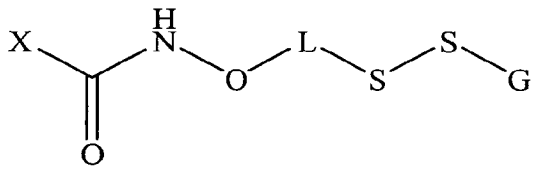
wherein X can be a residue of any macromolecule described herein and L can be a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof,

with a second thiolated compound having at least one SH group in the presence of an oxidant,

wherein the first thiolated compound and second thiolated compound are the same or different compounds.

In one aspect, X is a residue of any modified-glycosaminoglycan described herein. In another aspect, X can be a residue of hyaluronan. In one aspect, the second thiolated compound has the formula XVII. In a further aspect, the first and second thiolated compounds are the same compound.

The reaction between the thiolated compound having the formula XVII and the second thiolated compound produces a crosslinked compound having the fragment XVIII



XVIII

where X and L can be any macromolecule and linker, respectively, described herein. In one aspect, X is a modified-glycosaminoglycan described herein. In another aspect, X and G are a residue of hyaluronan.

b) Coupling Compounds via the Reaction between a Thiol Compound and a Thiol-Reactive Compound

In another aspect, described herein is a method for coupling two or more compounds by reacting a first thiolated compound having at least one SH group with at least one compound having at least one thiol-reactive electrophilic functional group. In one aspect, the compound has at least two-thiol reactive functional groups.

Any of the thiolated macromolecules described above or macromolecules that can be thiolated can be used in this aspect as the first thiolated compound. Two or more different macromolecules can be used in this method. For example, a second thiolated compound can be used in combination with the first thiolated

compound. In this aspect, the first and second thiolated compound can be the same or different compounds.

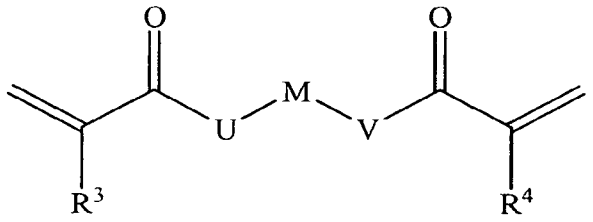
In one aspect, the first and second thiolated compound can be a polysaccharide. In this aspect, the polysaccharide is a sulfated-glycosaminoglycan including, but not limited to, chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, heparan sulfate, alginic acid, pectin, or carboxymethylcellulose.

In another aspect, the first thiolated compound is hyaluronan. In another aspect, the first thiolated compound has the formula XVII described above. In this aspect, X is a residue of hyaluronan and L is CH_2, CH_2CH_2 or $CH_2CH_2CH_2$. In another aspect, X is a residue of a modified-glycosaminoglycan.

In another aspect, the first thiolated compound has the formula X described above. In one aspect, Y in formula X is a modified-glycosaminoglycan.

In one aspect, the thiol-reactive compound contains one or more thiol-reactive electrophilic functional groups as defined above. In one aspect, the thiol-reactive compound has two electron-deficient vinyl groups, wherein the two electron-deficient vinyl groups are the same. In another aspect, the thiol-reactive compound is a diacrylate, a dimethacrylate, a diacrylamide, a dimethacrylamide, or a combination thereof. In another aspect, the thiol-reactive compound can be a dendrimer having a plurality of thiol-reactive groups. In one aspect, the thiol-reactive compound can have from 2 to 100, 2 to 90, 2 to 80, 2 to 70, 2 to 60, 2 to 50, 2 to 40, 2 to 30, 2 to 20 or 2 to 10 thiol-reactive groups.

In another aspect, the thiol-reactive compound has the formula XX



XX

wherein

R^3 and R^4 are, independently, hydrogen or lower alkyl;

U and V are, independently, O or NR^5, wherein R^5 is, independently, hydrogen or lower alkyl; and

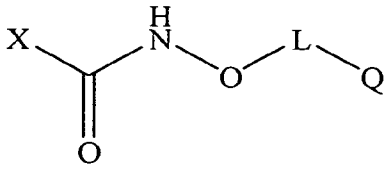
M is a polyalkylene group, a polyether group, a polyamide group, a polyimino group, a polyester, an aryl group, or a polythioether group.

In one aspect, R^3 and R^4 are hydrogen, U and V are oxygen, and M is a polyether group. In another aspect, R^3 and R^4 are hydrogen, U and V are NH, and M is a polyether group. In a further aspect, R^3 and R^4 are methyl, U and V are oxygen, and M is a polyether group. In another aspect, R^3 and R^4 are methyl, U and V are NH, and M is a polyether group.

In another aspect, the thiol-reactive compound is any bioactive agent described above containing at least one thiol-reactive electrophilic group. For example, Mitomycin C (MMC) can be converted to the corresponding acrylate (MMC-acrylate). MMC-acrylate can then be coupled with any of the thiolated macromolecules described herein.

In another aspect, the first thiolated compound has the formula X or XVII described above, wherein L is CH_2CH_2 or $CH_2CH_2CH_2$, and the thiol-reactive compound has the formula XX described above, wherein R^3 and R^4 are, independently, hydrogen or lower alkyl; U and V are, independently, O or NR^5, wherein R^5 is, independently, hydrogen or lower alkyl; and M is a polyether group.

In another aspect, described herein is a method for coupling a compound by reacting a first thiolated compound having at least one thiol-reactive electrophilic functional group with at least one compound having at least two thiol groups. Any of the thiolated macromolecules and thiol-reactive electrophilic functional groups described above can be used in this aspect. In one aspect, a thiol-reactive compound having at least one fragment having the formula XVI



XVI

wherein

X can be a residue of any macromolecule described herein;

Q is the thiol-reactive electrophilic functional group; and

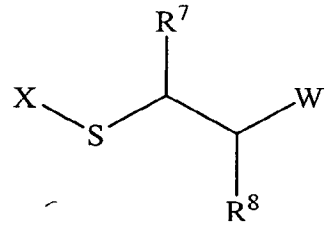
L can be a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof,

is reacted with at least one compound having at least two thiol groups.

In one aspect, when Q of formula XVI is thiol-reactive electrophilic functional group, X is a polysaccharide such as hyaluronan and L is CH_2CH_2 or $CH_2CH_2CH_2$. In another aspect, Q is an acrylate, a methacrylate, an acrylamide, or a methacrylamide.

In one aspect, examples of compounds having at least two thiol groups include, but are not limited to, propane-1,3-dithiol, polyethylene glycol-α,Ω-dithiol, *para*, *ortho*, or *meta*-bisbenzyl thiol, dithiothreitol, a peptide containing two or more cysteine residues, or dendrimeric thiols.

The compounds produced by coupling a thiolated compound with a compound having at least one thiol-reactive electrophilic functional group possess at least one fragment of the formula XXVII



XXVII

wherein

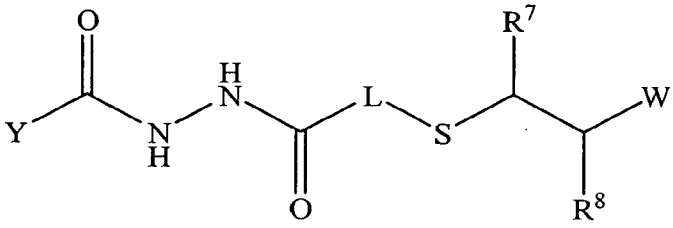
R^7 and R^8 are, independently, hydrogen or lower alkyl;

W is an electron-withdrawing group described above; and

X can be a residue of any macromolecule described herein.

In one aspect, X can be a residue of a polysaccharide such as hyaluronan or a sulfated-glycosaminoglycan. In another aspect, X can be a residue of a modified-glycosaminoglycan. In another aspect, R^7 is hydrogen and R^8 is hydrogen or methyl. In another aspect, X is a residue of a modified-glycosaminoglycan; R^7 is hydrogen; R^8 is hydrogen or methyl; and W is an ester group or an amide group.

In one aspect, the reaction product between the thiolated compound and thiol-reactive compound has at least one fragment having the formula XII



XII

wherein

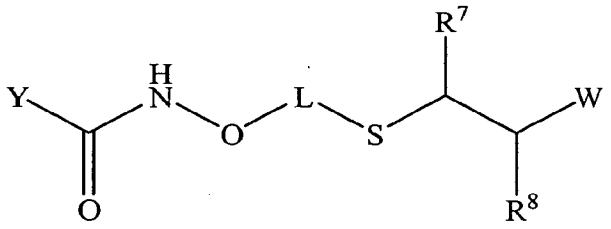
R^7 and R^8 can be, independently, hydrogen or lower alkyl;

W can be any electron-withdrawing group described herein;

Y can be a residue of any modified-glycosaminoglycan described herein; and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

In another aspect, the reaction product between the thiolated compound and thiol-reactive compound has at least one fragment having the formula XIII



XIII

wherein

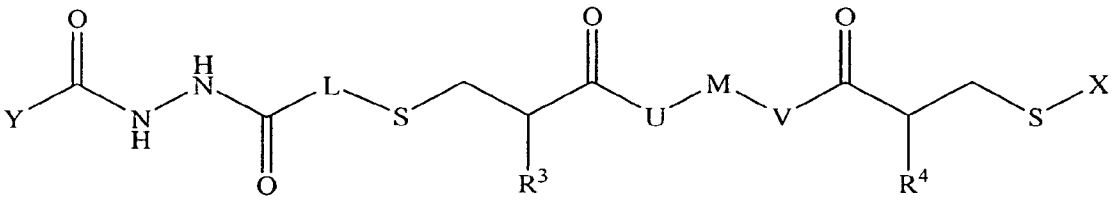
R^7 and R^8 can be, independently, hydrogen or lower alkyl;

W can be any electron-withdrawing group described herein;

Y can be a residue of any modified-glycosaminoglycan described herein; and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

In another aspect, the reaction product between the thiolated compound and thiol-reactive compound has at least one fragment having the formula XIV



XIV

wherein

R^3 and R^4 can be, independently, hydrogen or lower alkyl;

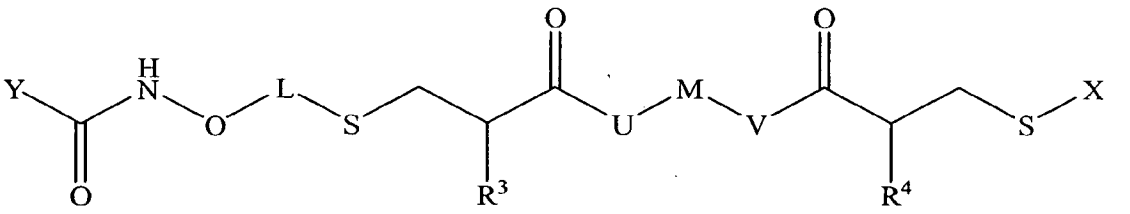
U and V can be, independently, O or NR^5, wherein R^5 is, independently, hydrogen or lower alkyl;

Y can be a residue of any modified-glycosaminoglycan described herein;

X can be a residue of any macromolecule described herein; and

L and M can be, independently, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

In another aspect, the reaction product between the thiolated compound and thiol-reactive compound has at least one fragment having the formula XV



XV

wherein

R^3 and R^4 can be, independently, hydrogen or lower alkyl;

U and V can be, independently, O or NR^5, wherein R^5 is, independently, hydrogen or lower alkyl;

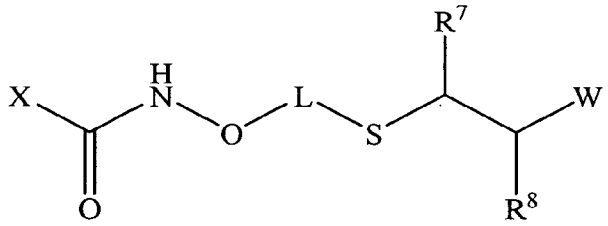
Y can be a residue of any modified-glycosaminoglycan described herein;

X can be a residue of any macromolecule described herein; and

L and M can be, independently, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino

group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

In another aspect, the reaction product between the thiolated compound and thiol-reactive compound has at least one fragment having the formula XXI



XXI

wherein

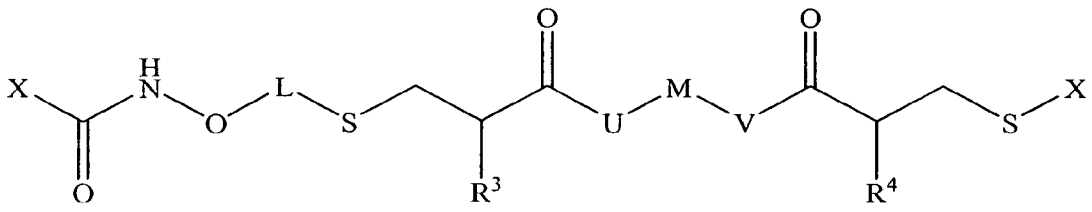
R^7 and R^8 can be, independently, hydrogen or lower alkyl;

W can be any electron-withdrawing group described herein;

X can be a residue of any macromolecule described herein; and

L can be a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

In another aspect, the reaction product between the thiolated compound and thiol-reactive compound has at least one fragment having the formula XXII



XXII

wherein

R^3 and R^4 can be, independently, hydrogen or lower alkyl;

U and V can be, independently, O or NR^5, wherein R^5 is, independently,

hydrogen or lower alkyl;

Y can be a residue of any modified-glycosaminoglycan described herein;

X can be a residue of any macromolecule described herein; and

L and M can be, independently, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

In one aspect, the reaction between the thiol reactive compound and thiol compound is generally conducted at a pH of from 7 to 12, 7.5 to 11, 7.5 to 10, or 7.5 to 9.5, or a pH of 8. In one aspect, the solvent used can be water (alone) or an aqueous containing organic solvent. In one aspect, when the mixed solvent system is used, a base such as a primary, secondary, or tertiary amine can used. In one aspect, an excess of thiol compound is used relative to the thiol-reactive compound in order to ensure that all of the thiol-reactive compound is consumed during the reaction. Depending upon the selection of the thiol reactive compound, the thiol compound, the pH of the reaction, and the solvent selected, coupling can occur from within minutes to several days. If the reaction is performed in the presence of an oxidant, such as air, the thiol compound can react with itself or another thiol compound via oxidative addition to form a disulfide linkage in addition to reacting with the thiol-reactive compound.

c) Crosslinking via Polycarbonyl Crosslinkers

In one aspect, a polycarbonyl crosslinker can react with any of the modified macromolecules described herein. The term "polycarbonyl crosslinker" is defined herein as a compound that possesses two or more groups represented by the formula C(O)R, where R is hydrogen, lower alkyl, or OR', where R' is a group that results in the formation of an activated ester. In one aspect, any of the hydrazide-modified

macromolecules and aminooxy-modified macromolecules can be crosslinked with a polyaldehyde. A polyaldehyde is a compound that has two or more aldehyde groups [C(O)H]. In one aspect, the polyaldehyde is a dialdehyde compound.

In one aspect, any compound possessing two or more aldehyde groups can be used as the polyaldehyde crosslinker. In one aspect, the polyaldehyde can be substituted or unsubstituted hydrocarbyl or substituted or unsubstituted heterohydrocarbyl. In another embodiment, the polyadlehyde can contain a polysaccharyl group or a polyether group. In a further aspect, the polyaldehyde can be a dendrimer or peptide. In one aspect, a polyether dialdehyde such as poly(ethylene glycol) propiondialdehyde (PEG) is useful in the compositions and methods described herein. PEG can be purchased from many commercial sources, such as Shearwater Polymers, Inc. (Huntsville, AL). In another aspect, the polyaldehyde is glutaraldehyde.

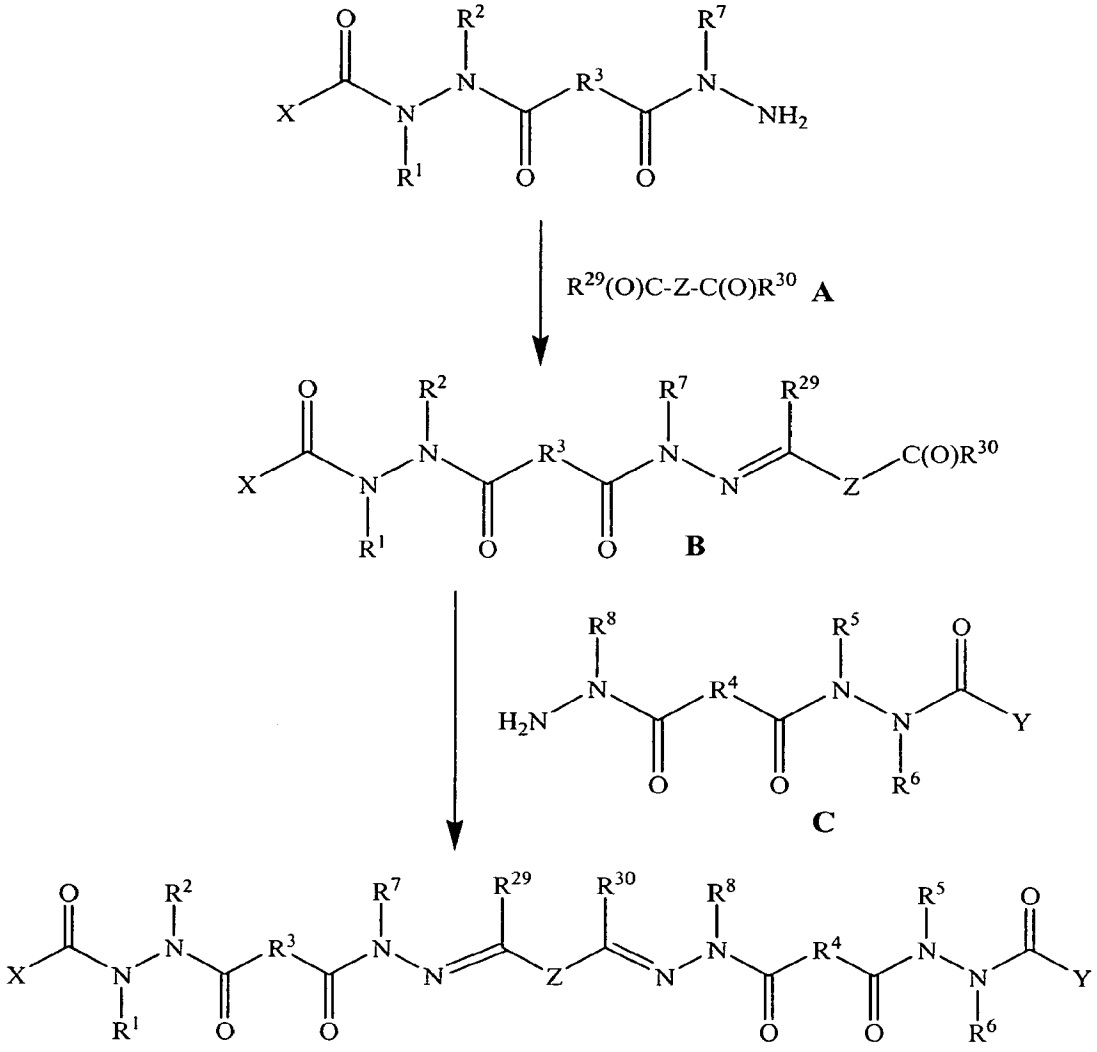
In another aspect, when the polycarbonyl compound is a polyaldehyde, the polyaldehyde can be prepared by the oxidation of terminal polyols or polyepoxides possessing two or more hydroxy or epoxy groups, respectively, using techniques known in the art.

The method of crosslinking generally involves reacting the modified macromolecule with the polycarbonyl crosslinker in the presence of a solvent. In one aspect, the carbonyl group of the polycarbonyl reacts with the hydrazide group or the amino group of the aminooxy group of the modified macromolecule to produce a new carbon-nitrogen double bond.

Scheme 3 depicts one aspect of using a dicarbonyl compound **A**, where R^{29} and R^{30} can be, independently, hydrogen, lower alkyl, or OR' as defined above, as a crosslinker. The carbonyl group of compound **B,** which is the result of one condensation reaction between a first hydrazide-modified macromolecule and the dicarbonyl, can react with the hydrazide group of a second hydrazide-modified

polysaccharide **C** to produce another carbon-nitrogen double bond, which results in the formation of a unit depicted in Formula V.

SCHEME 3



V

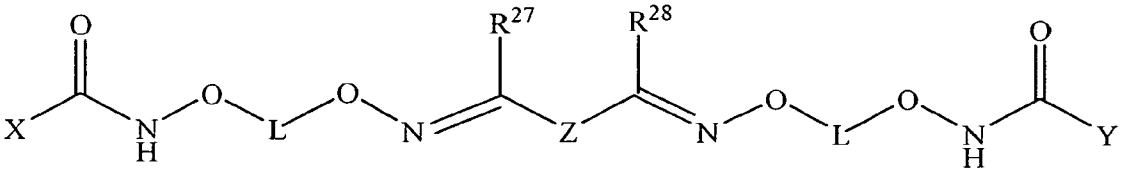
In view of Scheme 3, it is possible to crosslink two or more modified macromolecules to produce a matrix. Although the polycarbonyl crosslinker is intended to react with hydrazide groups or aminooxy groups on different modified

macromolecules, it is also possible that the polycarbonyl crosslinker can react with two or more hydrazide groups or aminooxy groups present on the same modified macromolecule

It also evident in Scheme 3 that the modified macromolecules can be different or the same. Thus, in one aspect, X and Y in formula V can be the same macromolecule residue. In another aspect, X and Y can be different macromolecule residues. In one aspect, X and Y are, independently, a residue of chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, heparan sulfate, alginic acid, pectin, or carboxymethylcellulose. In another aspect, X and Y are a residue of hyaluronan. In another aspect, X and/or Y are a residue of a modified-glycosaminoglycan.

In one aspect, when Y in formula V is a modified-glycosaminoglycan, Z can be a polyether. In another aspect, when Y in formula V is a modified-glycosaminoglycan, R^1, R^2, R^5, R^6, R^7, and R^8 are hydrogen. In another aspect, when Y in formula V is a modified-glycosaminoglycan, R^3 and R^4 can be an alkyl group such as, for example, $(CH_2)_n$, wherein n is from 1 to 20, 1 to 18, 1 to 16, 1 to 14, 1 to 12, 1 to 10, 1 to 8, 2 to 6, or 2 to 4. In another aspect, crosslinked macromolecules can be produced by reacting (1) a modified macromolecule comprising the reaction product between adipic dihydrazide and a modified-glycosaminoglycan and (2) a poly(ethylene glycol) propiondialdehyde. In another aspect, crosslinked macromolecules can be produced by reacting (1) a modified macromolecule comprising the reaction product between an aminooxy ether compound possessing two or more aminooxy groups and a macromolecule and (2) a poly(ethylene glycol) propiondialdehyde.

In another aspect, the reaction product between a polycarbonyl crosslinker and an aminooxy-modified macromolecule has at least one fragment having the formula VI



VI

wherein

X and Y can be a residue of any macromolecule described herein;

R^{27} and R^{28} can be, independently, hydrogen or lower alkyl; and

L and Z can be, independently, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

In one aspect, X and Y are a residue of a polysaccharide such as a sulfated-glycosaminoglycan or hylauronan. In another aspect, Y can be a modified-glycosaminoglycan.

In another aspect, one or more hydroxyl groups present on the macromolecule can be oxidized to the corresponding aldehyde, which then can undergo crosslinking with a hydrazide compound or an aminooxy ether compound. In one aspect, periodate can be used to oxidize the macromolecule.

The overall number of crosslinks and the number of different modified macromolecules that are cross linked together are dependent on the number of reactive carbonyl groups in the polycarbonyl crosslinker and dihydrazide groups or aminooxy groups present on the modified macromolecule. In one aspect, there is at a minimum at least one crosslink (*i.e.*, unit) having the formula V or VI. In one aspect, 1% to 100%, 10% to 90%, 30% to 80%, or 40% to 70% of the dihydrazide groups or aminooxy groups are crosslinked with the polycarbonyl crosslinker. In another aspect, the compound has from 10 to 10,000 units, 10 to 9,000 units, 10 to

8,000 units, 10 to 7,000 units, 10 to 6,000 units, 10 to 5,000 units, 10 to 4,000 units, 10 to 3,000 units, 10 to 2,000 units, or 10 to 1,000 units having the formula V or VI.

In one aspect, adipic dihydrazide (ADH) will crosslink when it modifies the uronic acid in 1%-99% of the glycosaminoglycan or 1-50%. In one aspect, modification of the carboxylic acid containing polysaccharide such as glycosaminoglycan (for example HA) can contain 10-90% or 20-80% or 30-70% or 40-60% or about 50% derivatization and the derivatized polysaccharide can contain greater than 10% or 20% or 30% or 40% or 50% or 60% or 70% or 80% or 90% or 99% crosslinking. For example, a hyaluronan (HA) with 5,000 disaccharide units (normal high MW HA) has 5,000 carboxylic acid groups available. A 1% modification means that there are 50 ADHs per HA molecule, 10% would be 500 ADH/HA, etc. Thus, even at low modification levels, there are numerous sites per modified GAG molecule to form crosslinks.

Any of the techniques and procedures for crosslinking polyaldehydes with polysaccharides disclosed in International publication no. WO 02/06373, which is incorporated by reference in its entirety, can be used in the methods described herein. In one aspect, after the reaction between the polycarbonyl crosslinker and the modified macromolecule is complete, the solvent present in the crosslinked macromolecule can be evaporated by any method known in the art such as air-drying, rotary evaporation at low pressure and/or lyophilization. In one aspect, at least 80%, at least 85%, at least 90%, at least 95%, and at least 98% of the solvent contained within the crosslinked macromolecule should evaporate.

In one aspect, the reaction solvent is water. In addition, small amounts of water miscible organic solvents, such as an alcohol or DMF or DMSO, can be used as well. In one aspect, crosslinking can be performed at room temperature, for example, 25 °C, but the cross-linking reaction can be performed within a range of temperatures from below 4 °C to above 90 °C but typically would be performed at between 4 °C and 60 °C, more typically between 4 °C and 50 °C, and more preferably at 4 °C or 30 °C or 37 °C. The reaction will also work at a variety of pHs

between, for example, pH from 3 to 10, or pH from 4 to 9, or pH from 5 to 8, or preferably at neutral pH.

4. Anti-adhesion Composites

In one aspect, described herein are composites comprising (1) a first compound comprising a first anti-adhesion compound covalently bonded to a first anti-adhesion support and (2) a first prohealing compound.

The term "anti-adhesion compound" as referred to herein is defined as any compound that prevents cell attachment, cell spreading, cell growth, cell division, cell migration, or cell proliferation. In one aspect, compounds that induce apoptosis, arrest the cell cycle, inhibit cell division, and stop cell motility can be used as the anti-adhesion compound. Examples of anti-adhesion compounds include, but are not limited to anti-cancer drugs, anti-proliferative drugs, PKC inhibitors, ERK or MAPK inhibitors, cdc inhibitors, antimitotics such as colchicine or taxol, DNA intercalators such as adriamycin or camptothecin, or inhibitors of PI3 kinase such as wortmannin or LY294002. In one aspect, the anti-adhesion compound is a DNA-reactive compound such as mitomycin C. In another aspect, any of the oligonucleotides disclosed in U.S. Patent No. 6,551,610, which is incorporated by reference in its entirety, can be used as the anti-adhesion compound. In another aspect, any of the anti-inflammatory drugs described below can be the anti-adhesion compound. Examples of anti-inflammatory compounds include, but are not limited to, methyl prednisone, low dose aspirin, medroxy progesterone acetate, and leuprolide acetate.

The term "anti-adhesion support" as referred to herein is defined as any compound that is capable of forming a covalent bond with the anti-adhesion compound that does not adhere to, spread, or proliferate cells. In one aspect, the anti-adhesion support is a hydrophilic, natural or synthetic polymer. Any of the polyanionic polysaccharides disclosed in U.S. Patent No. 6,521,223, which is incorporated by reference in its entirety, can be used as the anti-adhesion support. Examples of polyanionic polysaccharides include, but are not limited to, hyaluronan,

sodium hyaluronate, potassium hyaluronate, magnesium hyaluronate, calcium hyaluronate, carboxymethylcellulose, carboxymethyl amylose, or a mixture of hyaluronic acid and carboxymethylcellulose.

The formation of the first compound involves reacting the anti-adhesion compound with the anti-adhesion support to form a new covalent bond. In one aspect, the anti-adhesion compound possesses a group that is capable of reacting with the anti-adhesion support. The group present on the anti-adhesion compound that can react with the anti-adhesion support can be naturally-occurring or the anti-adhesion compound can be chemically modified to add such a group. In another aspect, the anti-adhesion support can be chemically modified so that it is more reactive with the anti-adhesion compound.

In one aspect, the first compound can be formed by crosslinking the anti-adhesion compound with the anti-adhesion support. In one aspect, the anti-adhesion compound and the anti-adhesion support each possess at least one hydrazide group or aminooxy group, which then can react with a crosslinker such as, for example, a polycarbonyl crosslinker having at least two hydrazide-reactive groups or at least two aminooxy-reactive groups. Any of the hydrazide-reactive groups, aminooxy-reactive groups, and polycarbonyl crosslinkers described above can be used in this aspect. In one aspect, the crosslinker is a polyethylene glycol dialdehyde. Additionally, any of the hydrazide-modified macromolecules and aminooxy-modified macromolecules described above can be used as the first anti-adhesion support.

In another aspect, the first compound can be formed by the oxidative coupling of the anti-adhesion compound with the anti-adhesion support. In one aspect, when the anti-adhesion compound and the anti-adhesion support each possess a thiol group, the anti-adhesion compound and the anti-adhesion support can react with one another in the presence of an oxidant to form a new disulfide bond. Any of the oxidants described above can be used in this aspect. Additionally, any of the thiolated hydrazide-modified macromolecules and thiolated aminooxy-modified

macromolecules described above can be used as first anti-adhesion support. For example, compounds having at least one fragment X or XVII can be used as the first anti-adhesion support.

The reaction between the anti-adhesion compound and the anti-adhesion support can be conducted in a buffer solution that is slightly basic. The amount of the anti-adhesion compound relative the amount of the anti-adhesion support can vary. In one aspect, the volume ratio of the anti-adhesion compound to the anti-adhesion support is from 99:1, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, or 1:99. In one aspect, the anti-adhesion compound and the anti-adhesion support react in air and are allowed to dry at room temperature. In this aspect, the dried material can be exposed to a second oxidant, such as hydrogen peroxide. The resultant compound can then be rinsed with water to remove any unreacted anti-adhesion compound, anti-adhesion support, and any unused oxidant. One advantage of preparing the first compound via the oxidative coupling methodology described herein is that coupling can occur in an aqueous media under physiologically benign conditions without the necessity of additional crosslinking reagents.

In another aspect, the first compound is produced by reacting the anti-adhesion support having at least one SH group with at least one anti-adhesion compound having at least one thiol-reactive electrophilic functional group. In one aspect, the anti-adhesion compound is mitomycin C having an acrylate group.

In another aspect, the first compound is produced by reacting the anti-adhesion support having at least one thiol-reactive electrophilic functional group with at least one anti-adhesion compound having at least two thiol groups. Any of the compounds described above that possess a thiol-reactive electrophilic functional group can be used in this aspect. For example, compounds having at least one fragment having the formula III or XVI can be used as the first adhesion support.

In one aspect, the reaction between the thiol reactive compound (anti-adhesion compound or the anti-adhesion support) and the thiol compound (anti-

adhesion compound or the anti-adhesion support) is generally conducted at a pH of from 7 to 12, 7.5 to 11, 7.5 to 10, or 7.5 to 9.5, or a pH of 8. In one aspect, the solvent used can be water (alone) or an aqueous solution containing an organic solvent. In one aspect, when the mixed solvent system is used, a base such as a primary, secondary, or tertiary amine can be used. In one aspect, an excess of thiol compound is used relative to the thiol-reactive compound in order to ensure that all of the thiol-reactive compound is consumed during the reaction. Depending upon the selection of the thiol reactive compound, the thiol compound, the pH of the reaction, and the solvent selected, coupling can occur from within minutes to several days. If the reaction is performed in the presence of an oxidant, such as air, the thiol compound can react with itself or another thiol compound via oxidative addition to form a disulfide linkage in addition to reacting with the thiol-reactive compound.

The composite can optionally contain unreacted (*i.e.*, free) anti-adhesion compound. The unreacted anti-adhesion compound can be the same or different anti-adhesion compound that is covalently bonded to the anti-adhesion support.

The composite is composed of a prohealing compound. The term "prohealing drug" as defined herein is any compound that promotes cell growth, cell proliferation, cell migration, cell motility, cell adhesion, or cell differentiation. In one aspect, the prohealing compound includes a protein or synthetic polymer. Proteins useful in the methods described herein include, but are not limited to, an extracellular matrix protein, a chemically-modified extracellular matrix protein, or a partially hydrolyzed derivative of an extracellular matrix protein. The proteins may be naturally occurring or recombinant polypeptides possessing a cell interactive domain. The protein can also be mixtures of proteins, where one or more of the proteins are modified. Specific examples of proteins include, but are not limited to, collagen, elastin, decorin, laminin, or fibronectin.

In one aspect, the synthetic polymer has at least one carboxylic acid group or the salt or ester thereof, which is capable of reacting with a hydrazide or an aminoooxy ether compound. In one aspect, the synthetic polymer comprises

glucuronic acid, polyacrylic acid, polyaspartic acid, polytartaric acid, polyglutamic acid, or polyfumaric acid.

In another aspect, the prohealing compound can be any of the supports disclosed in U.S. Patent No. 6,548,081 B2, which is incorporated by reference in its entirety. In one aspect, the prohealing compound includes cross-linked alginates, gelatin, collagen, cross-linked collagen, collagen derivatives, such as, succinylated collagen or methylated collagen, cross-linked hyaluronan, chitosan, chitosan derivatives, such as, methylpyrrolidone-chitosan, cellulose and cellulose derivatives such as cellulose acetate or carboxymethyl cellulose, dextran derivatives such carboxymethyl dextran, starch and derivatives of starch such as hydroxyethyl starch, other glycosaminoglycans and their derivatives, other polyanionic polysaccharides or their derivatives, polylactic acid (PLA), polyglycolic acid (PGA), a copolymer of a polylactic acid and a polyglycolic acid (PLGA), lactides, glycolides, and other polyesters, polyoxanones and polyoxalates, copolymer of poly(bis(p-carboxyphenoxy)propane)anhydride (PCPP) and sebacic acid, poly(1-glutamic acid), poly(d-glutamic acid), polyacrylic acid, poly(d1-glutamic acid), poly(1-aspartic acid), poly(d-aspartic acid), poly(d1-aspartic acid), polyethylene glycol, copolymers of the above listed polyamino acids with polyethylene glycol, polypeptides, such as, collagen-like, silk-like, and silk-elastin-like proteins, polycaprolactone, poly(alkylene succinates), poly(hydroxy butyrate) (PHB), poly(butylene diglycolate), nylon-2/nylon-6-copolyamides, polydihydropyrans, polyphosphazenes, poly(ortho ester), poly(cyano acrylates), polyvinylpyrrolidone, polyvinylalcohol, poly casein, keratin, myosin, and fibrin. In another aspect, highly cross-linked HA can be the prohealing compound.

In another aspect, the prohealing compound can be a polysaccharide. In one aspect, the polysaccharide has at least one group, such as a carboxylic acid group or the salt or ester thereof, that can react with a dihydrazide. In one aspect, the polysaccharide is a glycosaminoglycan (GAG). Any of the glycosaminoglycans

described above can be used in this aspect. In another aspect, the prohealing compound is hyaluronan.

The composite can optionally contain a second prohealing compound. In one aspect, the second prohealing compound can be a growth factor. Any substance or metabolic precursor which is capable of promoting growth and survival of cells and tissues or augmenting the functioning of cells is useful as a growth factor. Examples of growth factors include, but are not limited to, a nerve growth promoting substance such as a ganglioside, a nerve growth factor, and the like; a hard or soft tissue growth promoting agent such as fibronectin (FN), human growth hormone (HGH), a colony stimulating factor, bone morphogenic protein, platelet-derived growth factor (PDGF), insulin-derived growth factor (IGF-I, IGF-II), transforming growth factor-alpha (TGF-alpha), transforming growth factor-beta (TGF-beta), epidermal growth factor (EGF), fibroblast growth factor (FGF), interleukin-1 (IL-1), vascular endothelial growth factor (VEGF) and keratinocyte growth factor (KGF), dried bone material, and the like; and antineoplastic agents such as methotrexate, 5-fluorouracil, adriamycin, vinblastine, cisplatin, tumor-specific antibodies conjugated to toxins, tumor necrosis factor, and the like. The amount of growth factor incorporated into the composite will vary depending upon the growth factor and prohealing compound selected as well as the intended end-use of the composite.

Any of the growth factors disclosed in U.S. Patent No. 6,534,591 B2, which is incorporated by reference in its entirety, can be used in this aspect. In one aspect, the growth factor includes transforming growth factors (TGFs), fibroblast growth factors (FGFs), platelet derived growth factors (PDGFs), epidermal growth factors (EGFs), connective tissue activated peptides (CTAPs), osteogenic factors, and biologically active analogs, fragments, and derivatives of such growth factors. Members of the transforming growth factor (TGF) supergene family, which are multifunctional regulatory proteins. Members of the TGF supergene family include the beta transforming growth factors (for example, TGF-β1, TGF-β2, TGF-β3);

bone morphogenetic proteins (for example, BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8, BMP-9); heparin-binding growth factors (for example, fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF)); inhibins (for example, Inhibin A, Inhibin B); growth differentiating factors (for example, GDF-1); and Activins (for example, Activin A, Activin B, Activin AB).

Growth factors can be isolated from native or natural sources, such as from mammalian cells, or can be prepared synthetically, such as by recombinant DNA techniques or by various chemical processes. In addition, analogs, fragments, or derivatives of these factors can be used, provided that they exhibit at least some of the biological activity of the native molecule. For example, analogs can be prepared by expression of genes altered by site-specific mutagenesis or other genetic engineering techniques.

In another aspect, the addition of a crosslinker can be used to couple the first compound with the prohealing compound. In one aspect, when the first compound and the prohealing compound possess free thiol groups, a crosslinker having at least two thiol-reactive electrophilic groups can be used to couple the two compounds. Additionally, the crosslinker can couple two first compounds or two prohealing compounds.

In one aspect, the crosslinker is a thiol-reactive compound having two electron-deficient vinyl groups, wherein the two electron-deficient vinyl groups are the same. In another aspect, the thiol-reactive compound is a diacrylate, a dimethacrylate, a diacrylamide, a dimethacrylamide, or a combination thereof. In another aspect, the thiol-reactive compound has the formula XX discussed above.

The composites described herein can assume numerous shapes and forms depending upon the intended end-use. In one aspect, the composite is a laminate, a gel, a bead, a sponge, a film, a mesh, or a matrix. The procedures disclosed in U.S. Patent Nos. 6,534,591 B2 and 6,548,081 B2, which are incorporated by reference in their entireties, can be used for preparing composites having different forms.

In one aspect, the composite is a laminate. In one aspect, the laminate includes a first layer and a second layer, wherein (1) the first layer comprises a first compound comprising a first anti-adhesion compound covalently bonded to a first anti-adhesion support, wherein the first layer has a first surface and a second surface, and (2) the second layer comprises a first prohealing compound, wherein the second layer has a first surface and a second surface, wherein the first surface of the first layer is adjacent to the first surface of the second layer. In this aspect, the first layer is adjacent to the second layer. Depending upon the selection of the first compound and the prohealing compound, the first compound and the prohealing compound can either be covalently bonded to one another or merely in physical contact with one another without any chemical reaction occurring between the two compounds. In one aspect, the first compound and the prohealing compound possess free thiol groups, which can form new disulfide bonds in the presence of an oxidant.

In one aspect, a second layer of prohealing compound can be applied to a film of first layer. In one aspect, the width of the interface between the first and second layers can vary depending upon the casting time of the first layer. For example, if the casting time of the first layer is long, the width of the interface formed upon the application of the second layer will be decreased. Similarly, if the casting time of the first layer is short, a wider interface will be produced. By varying the width of the interface between the first and second layer, it is possible to create a gradient that will prevent cell growth either immediately (narrow interface) or gradually (wide interface). In another aspect, another layer of prohealing compound can be applied to the other surface of the first layer to produce a sandwich of first layer encased by prohealing compound. Figure 4 depicts one aspect of this sandwich laminate.

In one aspect, the composite can be molded into any desired shape prior to delivery to a subject. In another aspect, the second layer (prohealing compound) can be applied to a subject followed by the application of the first compound to the exposed second layer. In a further aspect, another layer containing the prohealing

compound can be applied to the exposed surface of the first layer. In this aspect, a sandwich laminate is formed *in situ* in the subject.

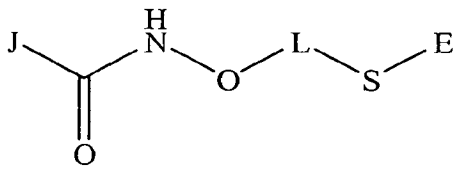
In one aspect, the first compound and prohealing compound can be used as a kit. For example, the first compound and prohealing compound are in separate syringes, with the contents being mixed using syringe-to-syringe techniques just prior to delivery to the subject. In this aspect, the first compound and prohealing compound can be extruded from the opening of the syringe by an extrusion device followed by spreading the mixture via spatula.

In another aspect, the first compound and the prohealing compound are in separate chambers of a spray can or bottle with a nozzle or other spraying device. In this aspect, the first compound and prohealing compound do not actually mix until they are expelled together from the nozzle of the spraying device.

5. Crosslinked Proteins

Described herein are methods for coupling a protein with another molecule using aminooxy ether compounds. In one aspect, a protein having at least one aminooxy-reactive group is reacted with a compound having at least one aminooxy group. In another aspect, a protein having at least one aminooxy group is reacted with a compound having at least one aminooxy -reactive group. In one aspect, the hydrazide-reactive group can be a –COOH group (or the salt or ester thereof), an aldehyde group, or a ketone group. The techniques disclosed in international publication nos. WO 02/06373 A1 and WO 02/090390 A1, which are incorporated by reference in their entireties, can be used in this aspect.

In one aspect, the coupled protein has at least one fragment having the formula XXIII



XXIII

wherein

J can be any protein residue;

L can be a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof; and

E can be a fluorescent tag, a radiolabel, a targeting moiety, a lipid, a peptide, a radionuclide chelator with a radionuclide, a spin-label, a PEG camouflage, a metal surface, a glass surface, a plastic surface, or a combination thereof.

The protein residue can be any protein that has at least one aminooxy-reactive group or at least one aminooxy group. Any of the protein known in the art capable of being modified with an aminooxy group can be used herein. In one aspect, the protein can be an extracellular matrix protein, a partially hydrolyzed extracellular matrix protein, or a chemically-modified extracellular matrix protein. In another aspect, the protein is collagen, elastin, decorin, laminin, or fibronectin.

In one aspect, E in formula XXIII is a reporter group. Examples of reporter groups include, but are not limited to, a chelated paramagnetic ion for MRI imaging, a ^{18}F-labelled compound having a thiol-reactive group for positron emission tomography, a fluorescent tag, a radiolabel, a targeting moiety, a lipid, a peptide, a radionuclide chelator with a radionuclide, a spin-label, a PEG camouflage, a glass surface, a plastic surface, or a combination thereof. Examples of spin labels include, but are not limited to, proxyl or doxyl groups. Examples of glass surfaces include, but are not limited to, glass silanized with an epoxy or activated ester or a thiol-reactive electrophilic functional group, beads, or coverslips. Examples of plastics include, but are not limited to, plasma-etched polypropylene or any other plastic material.

In another aspect, described herein is a kit including (1) a compound having at least one aminooxy group; (2) a condensing agent; (3) a buffer reagent; and (4) a purification column. In one aspect, the compound can be any compound having at

least one aminooxy group and at least one of the reporter groups described above. Use of the kit generally involves admixing components (1)-(3) together with a protein having at least one aminooxy -reactive group. Components (1)-(3) and the protein can be added in any order. After the protein and the compound having at least one aminooxy group have reacted with one another to produce the coupled protein, the coupled protein is then purified by passing the admixture containing the coupled protein through a purification column. Purification columns and techniques for using the same are known in the art.

B. Pharmaceutical Compositions

In one aspect, any of the compounds, composites, and compositions produced by the methods described above can include at least one bioactive agent defined above that is not covalently attached to the macromolecule. The resulting pharmaceutical composition can provide a system for sustained, continuous delivery of drugs and other biologically-active agents to tissues adjacent to or distant from the application site. The bioactive agent is capable of providing a local or systemic biological, physiological or therapeutic effect in the biological system to which it is applied. For example, the agent can act to control infection or inflammation, enhance cell growth and tissue regeneration, control tumor growth, act as an analgesic, promote anti-cell attachment, and enhance bone growth, among other functions. Additionally, any of the compounds, composites, and compositions described herein can contain combinations of two or more bioactive agents.

In one aspect, the bioactive agents can include substances capable of preventing an infection systemically in the biological system or locally at the defect site, as for example, anti-inflammatory agents such as, but not limited to, pilocarpine, hydrocortisone, prednisolone, cortisone, diclofenac sodium, indomethacin, 6∝-methyl-prednisolone, corticosterone, dexamethasone, prednisone, and the like; antibacterial agents including, but not limited to, penicillin, cephalosporins, bacitracin, tetracycline, doxycycline, gentamycin, chloroquine,

vidarabine, and the like; analgesic agents including, but not limited to, salicylic acid, acetaminophen, ibuprofen, naproxen, piroxicam, flurbiprofen, morphine, and the like; local anesthetics including, but not limited to, cocaine, lidocaine, benzocaine, and the like; immunogens (vaccines) for stimulating antibodies against hepatitis, influenza, measles, rubella, tetanus, polio, rabies, and the like; peptides including, but not limited to, leuprolide acetate (an LH-RH agonist), nafarelin, and the like. All compounds are available from Sigma Chemical Co. (Milwaukee, WI).

Additionally, a substance or metabolic precursor which is capable of promoting growth and survival of cells and tissues or augmenting the functioning of cells is useful, as for example, a nerve growth promoting substance such as a ganglioside, a nerve growth factor, and the like; a hard or soft tissue growth promoting agent such as fibronectin (FN), human growth hormone (HGH), a colony stimulating factor, bone morphogenic protein, platelet-derived growth factor (PDGF), insulin-derived growth factor (IGF-I, IGF-II), transforming growth factor-alpha (TGF-alpha), transforming growth factor-beta (TGF-beta), epidermal growth factor (EGF), fibroblast growth factor (FGF), interleukin-1 (IL-1), vascular endothelial growth factor (VEGF) and keratinocyte growth factor (KGF), dried bone material, and the like; and antineoplastic agents such as methotrexate, 5-fluorouracil, adriamycin, vinblastine, cisplatin, tumor-specific antibodies conjugated to toxins, tumor necrosis factor, and the like.

Other useful substances include hormones such as progesterone, testosterone, and follicle stimulating hormone (FSH) (birth control, fertility-enhancement), insulin, and the like; antihistamines such as diphenhydramine, and the like; cardiovascular agents such as papaverine, streptokinase and the like; anti-ulcer agents such as isopropamide iodide, and the like; bronchodilators such as metaproternal sulfate, aminophylline, and the like; vasodilators such as theophylline, niacin, minoxidil, and the like; central nervous system agents such as tranquilizer, B-adrenergic blocking agent, dopamine, and the like; antipsychotic agents such as risperidone, narcotic antagonists such as naltrexone, naloxone, buprenorphine; and

other like substances. All compounds are available from Sigma Chemical Co. (Milwaukee, WI).

The pharmaceutical compositions can be prepared using techniques known in the art. In one aspect, the composition is prepared by admixing a modified or crosslinked macromolecule described herein with a bioactive agent. The term "admixing" is defined as mixing the two components together so that there is no chemical reaction or physical interaction. The term "admixing" also includes the chemical reaction or physical interaction between the compound and the pharmaceutically-acceptable compound. Covalent bonding to reactive therapeutic drugs, e.g., those having reactive carboxyl groups, can be undertaken on the compound. For example, first, carboxylate-containing chemicals such as anti-inflammatory drugs ibuprofen or hydrocortisone-hemisuccinate can be converted to the corresponding N-hydroxysuccinimide (NHS) active esters and can further react with the NH_2 group of the dihydrazide-modified polysaccharide. Second, non-covalent entrapment of a bioactive agent in any of the compounds, composites, and compositions described herein is also possible. Third, electrostatic or hydrophobic interactions can facilitate retention of a bioactive agent in the compound, composite, and composition described herein. For example, the hydrazido group can non-covalently interact, e.g., with carboxylic acid-containing steroids and their analogs, and anti-inflammatory drugs such as Ibuprofen (2-(4-iso-butylphenyl) propionic acid). The protonated hydrazido group can form salts with a wide variety of anionic materials such as proteins, heparin or dermatan sulfates, oligonucleotides, phosphate esters, and the like.

It will be appreciated that the actual preferred amounts of bioactive compound in a specified case will vary according to the specific compound being utilized, the particular compositions formulated, the mode of application, and the particular situs and subject being treated. Dosages for a given host can be determined using conventional considerations, e.g. by customary comparison of the differential activities of the subject compounds and of a known agent, e.g., by means

of an appropriate conventional pharmacological protocol. Physicians and formulators, skilled in the art of determining doses of pharmaceutical compounds, will have no problems determining dose according to standard recommendations (Physicians Desk Reference, Barnhart Publishing (1999).

Pharmaceutical compositions described herein can be formulated in any excipient the biological system or entity can tolerate. Examples of such excipients include, but are not limited to, water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, vegetable oils such as olive oil and sesame oil, triglycerides, propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate can also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosol, cresols, formalin and benzyl alcohol.

Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH.

Molecules intended for pharmaceutical delivery can be formulated in a pharmaceutical composition. Pharmaceutical compositions can include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions can also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

The pharmaceutical composition can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be

treated. Administration can be topically (including ophthalmically, vaginally, rectally, intranasally).

Preparations for administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles, if needed for collateral use of the disclosed compositions and methods, include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles, if needed for collateral use of the disclosed compositions and methods, include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives can also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Formulations for topical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable.

Dosing is dependent on severity and responsiveness of the condition to be treated, but will normally be one or more doses per day, with course of treatment lasting from several days to several months or until one of ordinary skill in the art determines the delivery should cease. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates.

In one aspect, any of the compounds, composites, and compositions described herein can include living cells. Examples of living cells include, but are not limited to, fibroblasts, hepatocytes, chondrocytes, stem cells, bone marrow, muscle cells, cardiac myocytes, neuronal cells, or pancreatic islet cells.

C. Methods of Use

Any of the compounds, composites, compositions, and methods described herein can be used for a variety of uses related to drug delivery, small molecule delivery, wound healing, burn injury healing, and tissue regeneration. The disclosed compounds, composites, compositions, and methods are useful for situations which benefit from a hydrated, pericellular environment in which assembly of other matrix components, presentation of growth and differentiation factors, cell migration, or tissue regeneration are desirable.

The compounds, composites, and compositions described herein can be placed directly in or on any biological system without purification as it is composed of biocompatible materials. Examples of sites the compounds, composites, and compositions can be placed include, but not limited to, soft tissue such as muscle or fat; hard tissue such as bone or cartilage; areas of tissue regeneration; a void space such as periodontal pocket; surgical incision or other formed pocket or cavity; a natural cavity such as the oral, vaginal, rectal or nasal cavities, the cul-de-sac of the eye, and the like; the peritoneal cavity and organs contained within, and other sites into or onto which the compounds can be placed including a skin surface defect such as a cut, scrape or burn area. The compounds, composites, and compositions described herein can be biodegradable and naturally occurring enzymes will act to degrade them over time. Components of the compounds, composites, and compositions can be "bioabsorbable" in that the components of the compounds, composites, and compositions will be broken down and absorbed within the biological system, for example, by a cell, tissue and the like. Additionally, the compounds, composites, and compositions, especially the compounds, composites, and compositions that have not been rehydrated, can be applied to a biological system to absorb fluid from an area of interest.

The compounds, composites, and compositions described herein can be used in a number of different surgical procedures. In one aspect, the compounds, composites, and compositions can be used in any of the surgical procedures

disclosed in U.S. Patent Nos. 6,534,591 B2 and 6,548,081 B2, which are incorporated by reference in their entireties. In one aspect, the compounds, composites, and compositions described herein can be used in cardiosurgery and articular surgery; abdominal surgery where it is important to prevent adhesions of the intestine or the mesentery; operations performed in the urogenital regions where it is important to ward off adverse effects on the ureter and bladder, and on the functioning of the oviduct and uterus; and nerve surgery operations where it is important to minimize the development of granulation tissue. In surgery involving tendons, there is generally a tendency towards adhesion between the tendon and the surrounding sheath or other surrounding tissue during the immobilization period following the operation. In another aspect, the compounds, composites, and compositions described herein can be used to prevent adhesions after laparascopic surgery, pelvic surgery, oncological surgery, sinus and craniofacial surgery, ENT surgery, or in procedures involving spinal dura repair.

In another aspect, the compounds, composites, and compositions can be used in ophthalmological surgery. In ophthalmological surgery, a biodegradable implant could be applied in the angle of the anterior chamber of the eye for the purpose of preventing the development of synechiae between the cornea and the iris; this applies especially in cases of reconstructions after severe damaging events. Moreover, degradable or permanent implants are often desirable for preventing adhesion after glaucoma surgery and strabismus surgery.

In another aspect, the compounds, composites, and compositions can be used in the repair of tympanic membrane perforations (TMP). The tympanic membrane (TM) is a three-layer structure that separates the middle and inner ear from the external environment. These layers include an outer ectodermal portion composed of keratinizing squamous epithelium, an intermediate mesodermal fibrous component and an inner endodermal mucosal layer. This membrane is only 130 μm thick but provides important protection to the middle and inner ear structures and auditory amplification.

TMP is a common occurrence usually attributed to trauma, chronic otitis media or from PE tube insertion. Blunt trauma resulting in a longitudinal temporal bone fracture is classically associated with TMP. More common causes include a slap to the ear and the ill-advised attempt to clean an ear with a cotton swab (Q-tip™) or sharp instrument.

Any of the compounds, composites, and compositions described herein can be administered through the tympanic membrane without a general anesthetic and still provide enhanced wound healing properties. In one aspect, the compounds, composites, and compositions can be injected through the tympanic membrane using a cannula connected to syringe.

In another aspect, the compounds, composites, and compositions described herein can be used as a postoperative wound barrier following endoscopic sinus surgery. Success in functional endoscopic sinus surgery (FESS) is frequently limited by scarring, which narrows or even closes the surgically widened openings. Spacers and tubular stents have been used to temporarily maintain the opening, but impaired wound healing leads to poor long-term outcomes. The use of any compounds, composites, and compositions described herein can significantly decrease scar contracture following maxillary sinus surgery.

In another aspect, the compounds, composites, and compositions described herein can be used for the augmentation of soft or hard tissue. In another aspect, the compounds, composites, and compositions described herein can be used to coat implants. In another aspect, the compounds, composites, and compositions described herein can be used to treat aneurisms.

The compounds, composites, and compositions described herein can be used as a carrier and delivery device for a wide variety of releasable bioactive agents having curative or therapeutic value for human or non-human animals. Any of the bioactive agents described above can be used in this aspect. Many of these substances which can be carried by the compounds, composites, and compositions are discussed above.

Depending upon the selection of the bioactive agent, the bioactive agent can be present in the first compound or the prohealing compound. Included among pharmaceutically-acceptable compounds that are suitable for incorporation into the compounds, composites, and compositions described herein are therapeutic drugs, e.g., anti-inflammatory agents, anti-pyretic agents, steroidal and non-steroidal drugs for anti-inflammatory use, hormones, growth factors, contraceptive agents, antivirals, antibacterials, antifungals, analgesics, hypnotics, sedatives, tranquilizers, anti-convulsants, muscle relaxants, local anesthetics, antispasmodics, antiulcer drugs, peptidic agonists, sympathiomimetic agents, cardiovascular agents, antitumor agents, oligonucleotides and their analogues and so forth. The pharmaceutically-acceptable compound is added in pharmaceutically active amounts.

The rate of drug delivery depends on the hydrophobicity of the molecule being released. For example, hydrophobic molecules, such as dexamethazone and prednisone are released slowly from the compound as it swells in an aqueous environment, while hydrophilic molecules, such as pilocarpine, hydrocortisone, prednisolone, cortisone, diclofenac sodium, indomethacin, 6∝-methyl-prednisolone and corticosterone, are released quickly. The ability of the compound to maintain a slow, sustained release of steroidal anti-inflammatories makes the compounds described herein extremely useful for wound healing after trauma or surgical intervention.

In certain methods the delivery of molecules or reagents related to angiogenesis and vascularization are achieved. Disclosed are methods for delivering agents, such as VEGF, that stimulate microvascularization. Also disclosed are methods for the delivery of agents that can inhibit angiogenesis and vascularization, such as those compounds and reagents useful for this purpose disclosed in but not limited to United States Patent Nos 6,174,861 for "Methods of inhibiting angiogenesis via increasing *in vivo* concentrations of endostatin protein;" 6,086,865 for "Methods of treating angiogenesis-induced diseases and pharmaceutical compositions thereof;" 6,024,688 for "Angiostatin fragments and method of use;"

6,017,954 for "Method of treating tumors using O-substituted fumagillol derivatives;" 5,945,403 for "Angiostatin fragments and method of use;" 5,892,069 "Estrogenic compounds as anti-mitotic agents;" for 5,885,795 for "Methods of expressing angiostatic protein;" 5,861,372 for "Aggregate angiostatin and method of use;" 5,854,221 for "Endothelial cell proliferation inhibitor and method of use;" 5,854,205 for "Therapeutic antiangiogenic compositions and methods;" 5,837,682 for "Angiostatin fragments and method of use;" 5,792,845 for "Nucleotides encoding angiostatin protein and method of use;" 5,733,876 for "Method of inhibiting angiogenesis;" 5,698,586 for "Angiogenesis inhibitory agent;" 5,661,143 for "Estrogenic compounds as anti-mitotic agents;" 5,639,725 for "Angiostatin protein;" 5,504,074 for "Estrogenic compounds as anti-angiogenic agents;" 5,290,807 for "Method for regressing angiogenesis using o-substituted fumagillol derivatives;" and 5,135,919 for "Method and a pharmaceutical composition for the inhibition of angiogenesis" which are herein incorporated by reference for the material related to molecules for angiogenesis inhibition.

In one aspect, the pharmaceutically-acceptable compound is pilocarpine, hydrocortisone, prednisolone, cortisone, diclofenac sodium, indomethacin, 6∝-methyl-prednisolone, corticosterone, dexamethasone and prednisone. However, methods are also provided wherein delivery of a pharmaceutically-acceptable compound is for a medical purpose selected from the group of delivery of contraceptive agents, treating postsurgical adhesions, promoting skin growth, preventing scarring, dressing wounds, conducting viscosurgery, conducting viscosupplementation, engineering tissue.

In one aspect, the compounds, composites, and compositions described herein can be used for the delivery of living cells to a subject. Any of the living cells described above can be used in the aspect. In one aspect, the living cells are part of the prohealing compound. For example, when the composite is a laminate, the living cells are present in the prohealing layer.

In one aspect, the compounds, composites, and compositions can be used for the delivery of growth factors and molecules related to growth factors. Any of the growth factors described above are useful in this aspect. In one aspect, the growth factor is part of the prohealing compound.

In one aspect, described herein are methods for reducing or inhibiting adhesion of two tissues in a surgical wound in a subject by contacting the wound of the subject with any of the compounds, composites, and compositions described herein. Not wishing to be bound by theory, it is believed that the first compound will prevent tissue adhesion between two different tissues (*e.g.*, organ and skin tissue). It is desirable in certain post-surgical wounds to prevent the adhesion of tissues in order to avoid future complications. The second layer and optional third layer will promote healing of the tissues.

In another aspect, when the composite is laminate, the laminate includes a first layer of anti-adhesion compound/support and a second layer composed of a prohealing compound, wherein the laminate is wrapped around a tissue. For example, the laminate can be wrapped around a tendon, where the first layer is in contact with the tendon, and the second layer is in contact with surrounding muscle tissue. In this aspect, the laminate contributes a cylindrical anti-adhesion layer around the tendon, while healing of the tendon is promoted by the inner layer of the cylindrical material.

The compounds, composites, and compositions described herein provide numerous advantages. For example, the composites provide a post-operative adhesion barrier that is at least substantially resorbable and, therefore, does not have to be removed surgically at a later date. Another advantage is that the compounds, composites, and compositions are also relatively easy to use, are capable of being sutured, and tend to stay in place after it is applied.

In another aspect, described herein are methods for improving wound healing in a subject in need of such improvement by contacting any of the compounds, composites, and compositions described herein with a wound of a subject in need of

wound healing improvement. Also provided are methods to deliver at least one bioactive agent to a patient in need of such delivery by contacting any of the compounds, composites, and compositions described herein with at least one tissue capable of receiving said bioactive agent.

The disclosed compounds, composites, and compositions can be used for treating a wide variety of tissue defects in an animal, for example, a tissue with a void such as a periodontal pocket, a shallow or deep cutaneous wound, a surgical incision, a bone or cartilage defect, and the like. For example, the compounds, composites, and compositions described herein can be in the form of a hydrogel film. The hydrogel film can be applied to a defect in bone tissue such as a fracture in an arm or leg bone, a defect in a tooth, a cartilage defect in the joint, ear, nose, or throat, and the like. The hydrogel film composed of the compounds, composites, and compositions described herein can also function as a barrier system for guided tissue regeneration by providing a surface on or through which the cells can grow. To enhance regeneration of a hard tissue such as bone tissue, it is preferred that the hydrogel film provides support for new cell growth that will replace the matrix as it becomes gradually absorbed or eroded by body fluids.

The compounds, composites, and compositions described herein can be delivered onto cells, tissues, and/or organs, for example, by injection, spraying, squirting, brushing, painting, coating, and the like. Delivery can also be via a cannula, catheter, syringe with or without a needle, pressure applicator, pump, and the like. The compounds, composites, and compositions described herein can be applied onto a tissue in the form of a film, for example, to provide a film dressing on the surface of the tissue, and/or to adhere to a tissue to another tissue or hydrogel film, among other applications.

In one aspect, the compounds, composites, and compositions described herein are administered via injection. For many clinical uses, when the compounds and composites are in the form of a hydrogel film, injectable hydrogels are preferred for three main reasons. First, an injectable hydrogel could be formed into any

desired shape at the site of injury. Because the initial hydrogels can be sols or moldable putties, the systems can be positioned in complex shapes and then subsequently crosslinked to conform to the required dimensions. Second, the hydrogel would adhere to the tissue during gel formation, and the resulting mechanical interlocking arising from surface microroughness would strengthen the tissue-hydrogel interface. Third, introduction of an *in situ*-crosslinkable hydrogel could be accomplished using needle or by laparoscopic methods, thereby minimizing the invasiveness of the surgical technique.

The compounds, composites, and compositions described herein can be used to treat periodontal disease, gingival tissue overlying the root of the tooth can be excised to form an envelope or pocket, and the composition delivered into the pocket and against the exposed root. The compounds, composites, and compositions can also be delivered to a tooth defect by making an incision through the gingival tissue to expose the root, and then applying the material through the incision onto the root surface by placing, brushing, squirting, or other means.

When used to treat a defect on skin or other tissue, the compounds, composites, and compositions described herein can be in the form of a hydrogel film that can be placed on top of the desired area. In this aspect, the hydrogel film is malleable and can be manipulated to conform to the contours of the tissue defect.

The compounds, composites, and compositions described herein can be applied to an implantable device such as a suture, claps, prosthesis, catheter, metal screw, bone plate, pin, a bandage such as gauze, and the like, to enhance the compatibility and/or performance or function of an implantable device with a body tissue in an implant site. The compounds, composites, and compositions can be used to coat the implantable device. For example, the compounds, composites, and compositions could be used to coat the rough surface of an implantable device to enhance the compatibility of the device by providing a biocompatible smooth surface which reduces the occurrence of abrasions from the contact of rough edges with the adjacent tissue. The compounds, composites, and compositions can also be

used to enhance the performance or function of an implantable device. For example, when the compounds, composites, and compositions are a hydrogel film, the hydrogel film can be applied to a gauze bandage to enhance its compatibility or adhesion with the tissue to which it is applied. The hydrogel film can also be applied around a device such as a catheter or colostomy that is inserted through an incision into the body to help secure the catheter/colosotomy in place and/or to fill the void between the device and tissue and form a tight seal to reduce bacterial infection and loss of body fluid.

It is understood that the disclosed compounds, composites, and compositions can be applied to a subject in need of tissue regeneration. For example, cells can be incorporated into the composites described herein for implantation. Examples of subjects that can be treated with the compounds, composites, and compositions described herein include mammals such as mice, rats, cows or cattle, horses, sheep, goats, cats, dogs, and primates, including apes, chimpanzees, orangatangs, and humans. In another aspect, the compounds, composites, and compositions described herein can be applied to birds.

When being used in areas related to tissue regeneration such as wound or burn healing, it is not necessary that the disclosed compounds, composites, and compositions, and methods eliminate the need for one or more related accepted therapies. It is understood that any decrease in the length of time for recovery or increase in the quality of the recovery obtained by the recipient of the disclosed compounds, composites, and compositions, and methods has obtained some benefit. It is also understood that some of the disclosed compounds, composites, and compositions, and methods can be used to prevent or reduce fibrotic adhesions occurring as a result of wound closure as a result of trauma, such surgery. It is also understood that collateral affects provided by the disclosed compounds, composites, and compositions, and methods are desirable but not required, such as improved bacterial resistance or reduced pain etc.

It is understood that any given particular aspect of the disclosed compositions and methods can be easily compared to the specific examples and embodiments disclosed herein, including the non- polysaccharide based reagents discussed in the Examples. By performing such a comparison, the relative efficacy of each particular embodiment can be easily determined. Particularly preferred assays for the various uses are those assays which are disclosed in the Examples herein, and it is understood that these assays, while not necessarily limiting, can be performed with any of the compositions and methods disclosed herein.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, and methods described and claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric. There are numerous variations and combinations of reaction conditions, e.g., component concentrations, desired solvents, solvent mixtures, temperatures, pressures and other reaction ranges and conditions that can be used to optimize the product purity and yield obtained from the described process. Only reasonable and routine experimentation will be required to optimize such process conditions.

1. Synthesis of Carboxymethyl-HA (CM-HA)

High molecular weight HA (1.00 g)(1.5 MDa, Clear Solution) was suspended in 10 ml 50% (w/w) NaOH solution. After 4 h, 70 ml isopropanol was added. Then, a solution of 1.0 g of chloroacetic acid in 30 ml isopropanol was added into the HA/isopropanol suspension dropwise with magnetic stirring at ambient

temperature. After 24 h, the final suspension was collected by filtration. The solids were redissolved in 100 ml distilled water and the solution pH was adjusted to neutral pH by adding concentration HCl. This solution was then dialyzed against distilled water extensively, and then filtered again and lyophilized to give CM-HA as a white powder.

The resonance for the CH_2 peak of the carboxymethyl group was observed in the H^1 NMR at δ 4.05 (Figure 5). Integration relative to the *N*-acetyl methyl group indicated a degree of substitution of ca. 30% for this preparation. The use of lower concentrations of NaOH also resulted in detectable diagnostic resonances for the newly introduced methylene group of CM-HA.

2. Synthesis of CM-HA-DTPH

CM-HA (0.5 g) was dissolved in 50 ml of distilled water, and then 0.139 g DTP was added and the solution pH was adjusted to 4.75 by adding 0.1 N HCl. Next, 0.12 g EDCI was added into the solution with magnetic stirring, and the solution pH was maintained at 4.75 by adding 0.1 N HCl. After 2 h, 0.50 g DTT was added and the solution pH was increased to 8.5. After 12 h, the solution pH was adjusted to 3.5 by adding 1.0 N HCl, and the solution was dialyzed exhaustively against dilute HCl (pH 3.5) containing 100 mM NaCl, followed by dialysis against dilute HCl, pH 3.5. When extraneous solids are present, the solution was filtered and the resultant solution was lyophilized to give CM-HA-DTPH as a white powder.

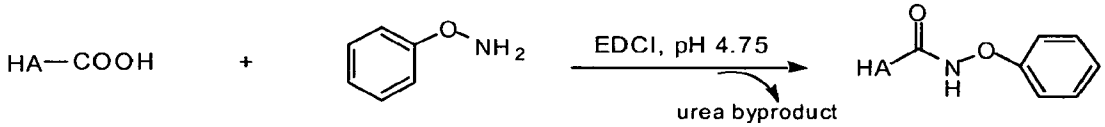
The peak at δ 4.08 was from the CH_2 peak of carboxymethyl group, and the peaks at δ 2.47 and 2.63 were from the two CH_2 groups of the DTP residue (Figure 6). The degree of DTPH substitution was determined by integration to be ca. 67%.

3. **The synthesis of an HA-Aminooxy ether**

Low molecular weight HA (190 KDa) (0.458 g, 1.145 mmol) was dissolved in 46 ml distilled water. Then, 0.5 g *O*-phenylhydroxylamine hydrochloride (OPH) (3.434 mmol) was added, and the solution pH was adjusted to 4.75 by adding 1.0 N

HCl. Next, 0.11 g EDCI (0.057 mmol) was added under magnetic stirring, and the solution pH was maintained at 4.75 for 4 h by adding 0.1 N HCl. The solution was dialyzed extensively against 100 mM NaCl, followed by dialysis against distilled water. After that the solution was filtered to remove extraneous solids and lyophilized to give the HA-OPH product as a white powder.

The peaks at δ 7.30 (2 protons) and 7.05 (3 protons) (Figure 7) were from the phenyl group, and the degree of substitution was found to be ca. 21% based on integration of the proton NMR resonances. Expected resonances for N-acetylurea-modified hyaluronan were detected at δ 1.10 and δ 2.78.



What is claimed:

1. A modified-glycosaminoglycan, wherein the modified-glycosaminoglycan comprises a glycosaminoglycan comprising at least one hydroxyl group chemically substituted with a hydrazide-reactive group or an aminooxy-reactive group.

2. The modified-glycosaminoglycan of claim 1, wherein the glycosaminoglycan comprises chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, or heparan sulfate.

3. The modified-glycosaminoglycan of claim 1, wherein the glycosaminoglycan comprises hyaluronan.

4. The modified-glycosaminoglycan of claim 3, wherein at least one primary C-6 hydroxyl group of a N-acetyl-glucosamine residue is substituted with the hydrazide-reactive group or the aminooxy-reactive group.

5. The modified-glycosaminoglycan of claim 4, wherein at least one secondary hydroxyl group is substituted with the hydrazide-reactive group or the aminooxy-reactive group.

6. The modified-glycosaminoglycan of claim 4, wherein from one primary C-6 hydroxyl group of the N-acetyl-glucosamine residue to 100 % of the primary C-6 hydroxyl groups of the N-acetyl-glucosamine residue are substituted with the hydrazide-reactive group or the aminooxy-reactive group.

7. The modified-glycosaminoglycan of claim 1, wherein the hydroxyl group comprises a primary C-6 hydroxyl group of the non-uronic acid sugar component of the repeating disaccharide of the glycosaminoglycan.

8. The modified-glycosaminoglycan of claim 1, wherein the hydrazide-reactive group or the aminooxy-reactive group comprises a carboxylic group or the salt or ester thereof.

9. The modified-glycosaminoglycan of claim 1, wherein the hydrazide-reactive group or the aminooxy-reactive group comprises the formula -L-CO_2H or the salt or ester thereof, wherein L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

10. The modified-glycosaminoglycan of claim 9, wherein L comprises a polyalkylene group having the formula $(CH_2)_n$, wherein n is from 1 to 10.

11. The modified-glycosaminoglycan of claim 1, wherein the hydrazide-reactive group or the aminooxy-reactive group comprises -CH_2CO_2H or the salt or ester thereof.

12. The modified-glycosaminoglycan of claim 1, wherein the modified-glycosaminoglycan comprises hyaluronan with at least one primary C-6 hydroxyl group of the N-acetyl-glucosamine residue substituted with -CH_2CO_2H or the salt or ester thereof.

13. The modified-glycosaminoglycan of claim 12, wherein at least one secondary hydroxyl group is substituted with the hydrazide-reactive group or the aminooxy-reactive group.

14. A method for making a modified-glycosaminoglycan, comprising (a) reacting a glycosaminoglycan with a base to produce deprotonated-glycosaminoglycan, and (b) reacting the deprotonated-glycosaminoglycan with a compound comprising at least one hydrazide-reactive group or aminooxy-reactive group.

15. The method of claim 14, wherein the base comprises a hydroxide, an alkoxide, a carbonate, a phosphate, or an amine.

16. The method of claim 14, wherein the compound is capable of reacting with an alkoxide.

17. The method of claim 14, wherein the compound comprises a leaving group.

18. The method of claim 14, wherein the compound comprises LG-L-G, wherein LG comprises a leaving group; L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof; and G comprises a hydrazide-reactive group or an aminooxy-reactive group.

19. The method of claim 18, wherein LG comprises a halogen.

20. The method of claim 18, wherein L comprises a polyalkylene group having the formula $(CH_2)_n$, wherein n is from 1 to 10.

21. The method of claim 20, wherein n is 1.

22. The method of claim 14, wherein G comprises a CO_2H group or the salt or ester thereof.

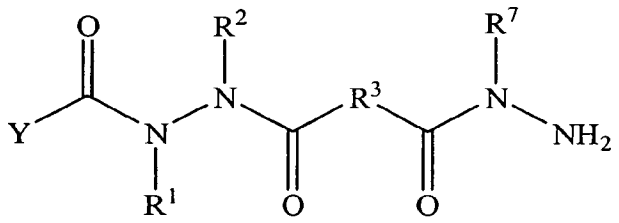
23. The method of claim 18, wherein the glycosaminoglycan comprises hyaluronan and the compound comprises $LGCH_2CO_2H$ or the salt or ester thereof, wherein LG comprises a halogen or OR^{21}, wherein R^{21} comprises mesylate, tosylate, or triflate.

24. A modified-glycosaminoglycan made by the process of claims 14-23.

25. A compound comprising the modified-glycosaminoglycan of claims 1-11 and 24, wherein the modified-glycosaminoglycan comprises at least one hydrazide group, at least one aminooxy group, or at least one hydrazide group and at least one aminooxy group.

26. The compound of claim 25, wherein the compound comprises two or more hydrazide groups.

27. The compound of claim 25, wherein the compound comprises at least one unit comprising the formula I



I

wherein

Y comprises a residue of the modified-glycosaminoglycan of claims 1-11 and 24; and

R^1, R^2, R^3, and R^7 comprise, independently, hydrogen, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, or a polyether group, wherein R^3 is not hydrogen.

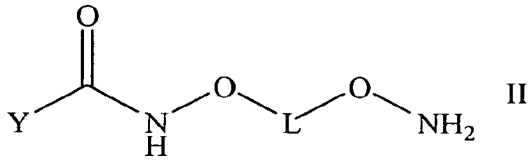
28. The compound of claim 27, wherein R^1, R^2, and $R^7$are hydrogen.

29. The compound of claim 27, wherein R^3 comprises an alkyl group.

30. The compound of claim 27, wherein R^3 comprises $(CH_2)_n$, wherein n is from 1 to 20.

31. The compound of claim 30, wherein n is from 2 to 4.

32. The compound of claim 25, wherein the compound comprises at least one unit comprising the formula II



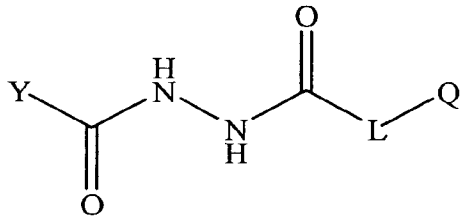
II

wherein

Y comprises a residue of the modified-glycosaminoglycan of claims 1-11 and 24; and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

33. The compound of claim 25, wherein the compound comprises at least one fragment comprising the formula III



III

wherein

Y comprises a residue of the modified-glycosaminoglycan of claims claims 1- 11 and 24;

Q comprises a residue of a bioactive agent, SH group or a thiol-reactive electrophilic functional group; and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

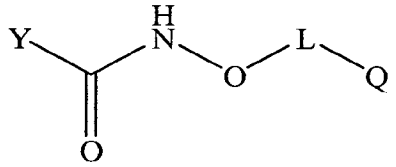
34. The compound of claim 33, wherein when Q is a thiol-reactive electrophilic functional group, the thiol-reactive electrophilic functional group comprises an electron-deficient vinyl group.

35. The compound of claim 34, wherein the electron-deficient vinyl group comprises a nitro group, a cyano group, an ester group, an aldehyde group, a keto group, a sulfone group, or an amide group.

36. The compound of claim 33, wherein when Q comprises a thiol-reactive electrophilic functional group, the thiol-reactive electrophilic functional group comprises an acrylate group.

37. The compound of claim 33, wherein L comprises CH_2, CH_2CH_2, or $CH_2CH_2CH_2$ and Q comprises a SH group.

38. The compound of claim 25, wherein the compound comprises at least one fragment comprising the formula IV



IV

wherein

Y comprises a residue of a modified-glycosaminoglycan of claims 1-11 and 24;

Q comprises a residue of a bioactive agent, an aminooxy group, SH group or a thiol-reactive electrophilic functional group; and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

39. The compound of claim 38, wherein when Q comprises a thiol-reactive electrophilic functional group, the thiol-reactive electrophilic functional group comprises an electron-deficient vinyl group.

40. The compound of claim 39, wherein the electron-deficient vinyl group comprises a nitro group, a cyano group, an ester group, an aldehyde group, a keto group, a sulfone group, or an amide group.

41. The compound of claim 38, wherein when Q comprises a thiol-reactive electrophilic functional group, the thiol-reactive electrophilic functional group comprises an acrylate group.

42. The compound of claim 38, wherein Q comprises a SH group.

43. The compound of claim 38, wherein L comprises a polyalkylene group comprising the formula $(CH_2)_n$, wherein n is from 1 to 10.

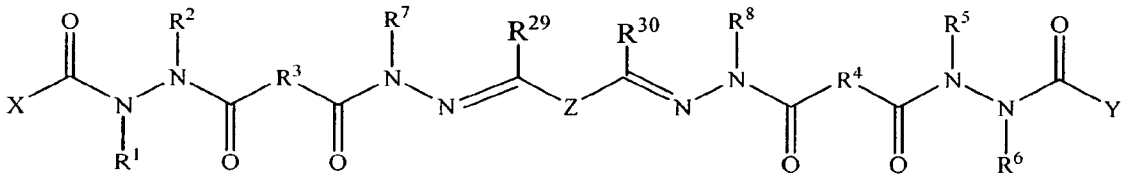
44. The compound of claim 43, wherein n is from 1 to 4.

45. A method for making a compound comprising reacting the modified-glycosaminoglycan of claims 1- 11 and 24 with a hydrazide compound.

46. A method for making a compound comprising reacting the modified-glycosaminoglycan of claims 1- 11 and 24 with an aminooxy ether compound.

47. The compounds produced by the methods of claims 45 or 46.

48. A compound comprising at least one fragment comprising the formula V



V

wherein

Y comprises a residue of the modified-glycosaminoglycan of claims 1-11 and 24;

X comprises a residue of a macromolecule;

R^{29} and R^{30} comprise, independently, hydrogen or lower alkyl; and

Z, R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 comprise, independently, hydrogen, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof, wherein Z, R^3, and R^4 are not hydrogen.

49. The compound of claim 48, wherein the macromolecule comprises an oligonucleotide, a nucleic acid or a metabolically stabilized analogue thereof, a polypeptide, a lipid, a glycoprotein, a glycolipid, or a pharmaceutically-acceptable compound.

50. The compound of claim 48, wherein the macromolecule comprises a polysaccharide, a protein, or a synthetic polymer.

51. The compound of claim 50, wherein the macromolecule comprises a polysaccharide, wherein the polysaccharide comprises a sulfated-glycosaminoglycan.

52. The compound of claim 48, wherein the macromolecule comprises chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, heparan sulfate, alginic acid, pectin, or carboxymethylcellulose.

53. The compound of claim 48, wherein the macromolecule comprises hyaluronan.

54. The compound of claim 48, wherein Z comprises a polyether.

55. The compound of claim 48, wherein R^1, R^2, R^5, R^6, R^7, and R^8 are hydrogen.

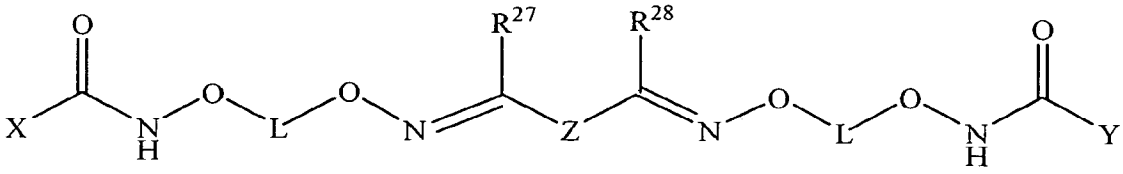
56. The compound of claim 48, wherein R^3 and R^4 comprise an alkyl group.

57. The compound of claim 48, wherein R^3 and R^4 comprise $(CH_2)_n$, wherein n is from 1 to 20.

58. The compound of claim 57, wherein n is from 2 to 4.

59. The compound of claim 48, wherein the compound comprises from 10 to 10,000 units having the formula V.

60. A compound comprising at least one fragment comprising the formula VI



VI

wherein

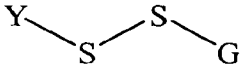
X and Y comprises a residue of a macromolecule;

R^{27} and R^{28} comprise, independently, hydrogen or lower alkyl; and

L and Z comprise, independently, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

61. A method for producing a compound comprising reacting (1) the compound of claims 25-44 or 47 with (2) a polycarbonyl crosslinker.

62. A compound comprising at least one fragment comprising the formula VII



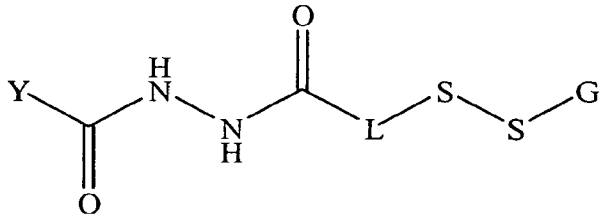
VII

wherein

Y comprises a residue of a modified-glycosaminoglycan of claims 1-11 and 24, and

G comprises a residue of a thiolated compound.

63. A compound comprising at least one fragment comprising the formula VIII



VIII

wherein

Y comprises a residue of a modified-glycosaminoglycan of claims 1-11 and 24;

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof; and

G comprises a residue of a thiolated compound.

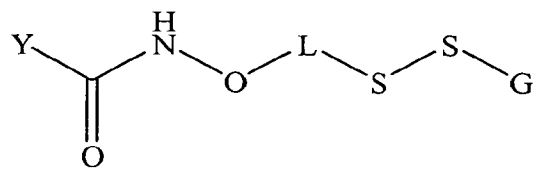
64. The compound of claim 63, wherein L comprises CH_2, CH_2CH_2, or $CH_2CH_2CH_2$.

65. The compound of claim 63, wherein G comprises a polysaccharide residue.

66. The compound of claim 63, wherein G comprises a sulfated-glycosaminoglycan residue.

67. The compound of claim 63, wherein G comprises a residue of chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, heparan sulfate, alginic acid, pectin, or carboxymethylcellulose, or hyaluronan.

68. A compound comprising at least one fragment comprising the formula IX



IX

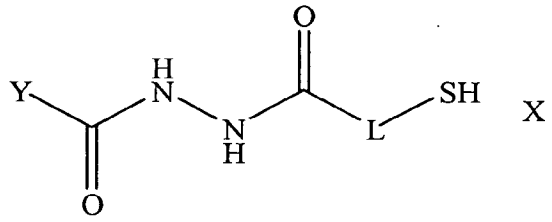
wherein

Y comprises a residue of a modified-glycosaminoglycan of claims 1-11 and 24;

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof; and

G comprises a residue of a thiolated compound.

69. A method for coupling two or more thiolated compounds, comprising reacting a first thiolated compound having the formula X



X

wherein

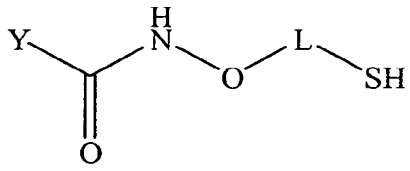
Y comprises a residue of a modified-glycosaminoglycan of claims 1-11 and 24, and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof,

with a second thiolated compound having at least one SH group in the presence of an oxidant,

wherein the first thiolated compound and second thiolated compound are the same or different compounds.

70. A method for coupling two or more thiolated compounds, comprising reacting a first thiolated compound having the formula XI



XI

wherein

Y comprises a residue of a modified-glycosaminoglycan of claims 1-11 and 24, and

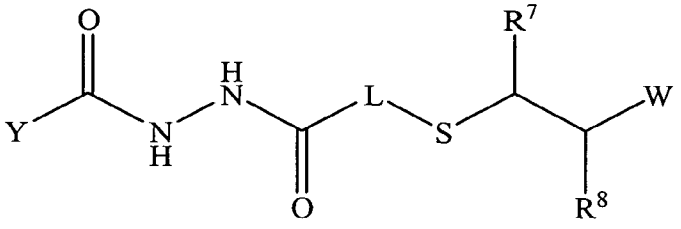
L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof,

with a second thiolated compound having at least one SH group in the presence of an oxidant,

wherein the first thiolated compound and second thiolated compound are the same or different compounds.

71. The compounds produced by the methods of claims 70 or 71.

72. A compound comprising at least one fragment comprising the formula XII



XII

wherein

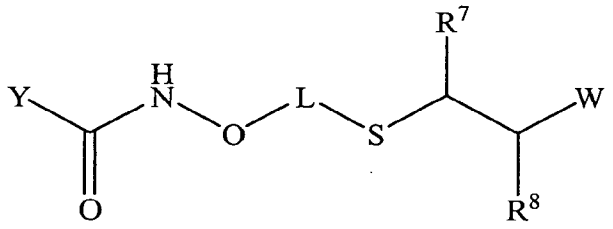
R^7 and R^8 comprise, independently, hydrogen or lower alkyl;

W comprises an electron-withdrawing group;

Y comprises a residue of a modified-glycosaminoglycan of claims 1-11 and 24; and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

73. A compound comprising at least one fragment comprising the formula XIII



XIII

wherein

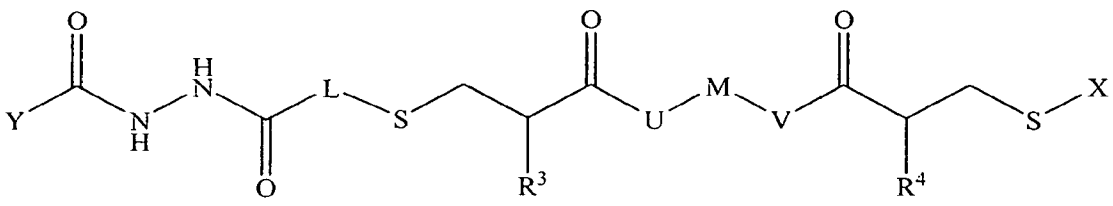
R^7 and R^8 comprise, independently, hydrogen or lower alkyl;

W comprises an electron-withdrawing group;

Y comprises a residue of a modified-glycosaminoglycan of claims 1-11 and 24; and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

74. A compound comprising at least one fragment comprising the formula XIV



XIV

wherein

R^3 and R^4 comprise, independently, hydrogen or lower alkyl;

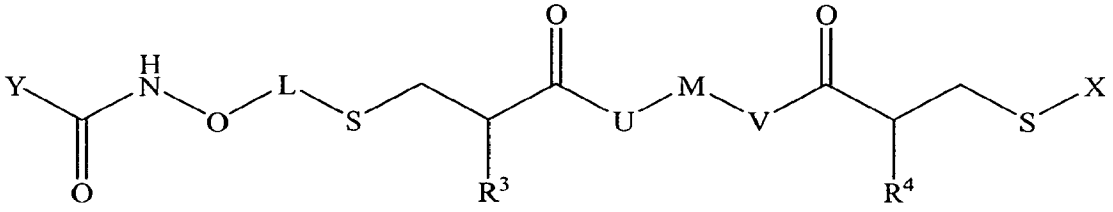
U and V comprise, independently, O or NR^5, wherein R^5 is, independently, hydrogen or lower alkyl;

Y comprises a residue of a modified-glycosaminoglycan of claims 1-11 and 24;

X comprises a residue of a macromolecule; and

L and M comprise, independently, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

75. A compound comprising at least one fragment comprising the formula XV



XV

wherein

R^3 and R^4 comprise, independently, hydrogen or lower alkyl;

U and V comprise, independently, O or NR^5, wherein R^5 is, independently, hydrogen or lower alkyl;

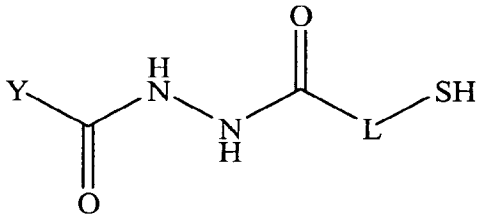
Y comprises a residue of a modified-glycosaminoglycan of claims 1-11 and 24;

X comprises a residue of a macromolecule; and

L and M comprise, independently, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a

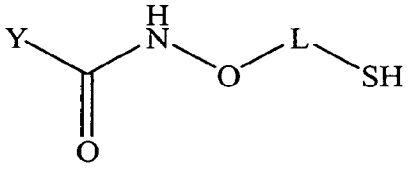
polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

76. A method for making a compound, comprising reacting a first thiolated compound comprising at least one fragment having the formula X or XI



X

or



XI

wherein

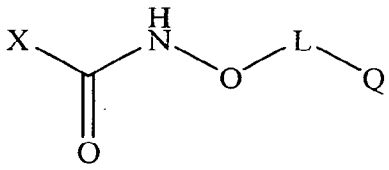
Y comprises a residue of a modified-glycosaminoglycan of claims 1-11 and 24, and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof,

with at least one compound having at least one thiol-reactive electrophilic functional group.

77. A compound made by the method of claim 76.

78. A compound comprising at least one fragment comprising the formula XVI



XVI

wherein

X comprises a residue of a macromolecule;

Q comprises a residue of a bioactive agent, an aminooxy group, a SH group, or a thiol-reactive electrophilic functional group; and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

79. The compound of claim 78, wherein the macromolecule comprises an oligonucleotide, a nucleic acid o r a metabolically stabilized analogue thereof, a polypeptide, a lipid, a glycoprotein, a glycolipid, or a pharmaceutically-acceptable compound.

80. The compound of claim 78, wherein the macromolecule comprises a polysaccharide, a protein, or a synthetic polymer.

81. The compound of claim 78, wherein the macromolecule comprises a polysaccharide, wherein the polysaccharide comprises a sulfated-glycosaminoglycan.

82. The compound of claim 78, wherein the macromolecule comprises a synthetic polymer, wherein the synthetic polymer comprises glucuronic acid, polyacrylic acid, polyaspartic acid, polytartaric acid, polyglutamic acid, or polyfumaric acid.

83. The compound of claim 78, wherein the macromolecule comprises a protein, wherein the protein comprises a naturally-occurring protein or a recombinant protein.

84. The compound of claim 78, wherein the macromolecule comprises a protein, wherein the protein comprises an extracellular matrix protein, a chemically-

modified extracellular matrix protein, or a partially hydrolyzed derivative of an extracellular matrix protein.

85. The compound of claim 78, wherein the macromolecule comprises a protein, wherein the protein comprises collagen, elastin, decorin, laminin, or fibronectin.

86. The compound of claim 78, wherein the macromolecule comprises chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, heparan sulfate, alginic acid, pectin, or carboxymethylcellulose.

87. The compound of claim 78, wherein the macromolecule comprises hyaluronan.

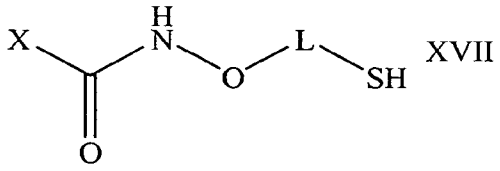
88. The compound of claim 78, wherein when Q comprises a thiol-reactive electrophilic functional group, the thiol-reactive electrophilic functional group comprises an electron-deficient vinyl group.

89. The compound of claim 88, wherein the electron-deficient vinyl group comprises a nitro group, a cyano group, an ester group, an aldehyde group, a keto group, a sulfone group, or an amide group.

90. The compound of claim 78, wherein when Q comprises a thiol-reactive electrophilic functional group, the thiol-reactive electrophilic functional group comprises an acrylate group.

91. The compound of claim 78, wherein when Q comprises a bioactive agent, the bioactive agent comprises a dye, a probe, a nucleic acid, an enzyme, an oligonucleotide, a label, a protein, a polypeptide, a lipid, a glycoprotein, a glycolipid, or a pharmaceutically-acceptable compound.

92. A method for coupling two or more thiolated compounds, comprising reacting a first thiolated compound having the formula XVII



XVII

wherein

X comprises a residue of a macromolecule, and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof,

with a second thiolated compound having at least one SH group in the presence of an oxidant,

wherein the first thiolated compound and second thiolated compound are the same or different compounds.

93. The method of claim 92, wherein the macromolecule comprises an oligonucleotide, a nucleic acid or a metabolically stabilized analogue thereof, a polypeptide, a lipid, a glycoprotein, a glycolipid, or a pharmaceutically-acceptable compound.

94. The method of claim 92, wherein the macromolecule comprises a polysaccharide, a protein, or a synthetic polymer.

95. The method of claim 94, wherein the macromolecule comprises a polysaccharide, wherein the polysaccharide comprises a sulfated-glycosaminoglycan.

96. The method of claim 92, wherein the macromolecule comprises chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, heparan sulfate, alginic acid, pectin, or carboxymethylcellulose.

97. The method of claim 92, wherein the macromolecule comprises hyaluronan.

98. The method of claim 92, wherein the second thiolated compound comprises a macromolecule comprising an oligonucleotide, a nucleic acid or a metabolically stabilized analogue thereof, a polypeptide, a lipid, a glycoprotein, a glycolipid, or a pharmaceutically-acceptable compound.

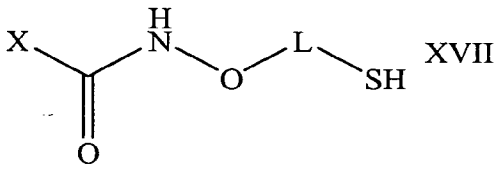
99. The method of claim 92, wherein the second thiolated compound comprises a polysaccharide having at least one SH group.

100. The method of claim 92, wherein the second thiolated compound comprises a sulfated-glycosaminoglycan having at least one SH group.

101. The method of claim 92, wherein the second thiolated compound comprises chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, heparan sulfate, alginic acid, pectin, or carboxymethylcellulose, or hyaluronan, wherein each compound has at least one SH group.

102. The method of claim 92 wherein the second thiolated compound comprises a thiolated protein.

103. The method of claim 92, wherein the second thiolated compound has the formula XVII



XVII

wherein

X comprises a residue of a macromolecule, and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an

aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof,

wherein the macromolecule residue in the first and second thiolated compounds is the same or different.

104. The method of claim 103, wherein the macromolecule comprises an oligonucleotide, a nucleic acid or a metabolically stabilized analogue thereof, a polypeptide, a lipid, a glycoprotein, a glycolipid, or a pharmaceutically-acceptable compound.

105. The method of claim 103, wherein the macromolecule comprises a polysaccharide, a protein, or a synthetic polymer.

106. The method of claim 92, wherein the first thiolated compound and the second thiolated compound are different.

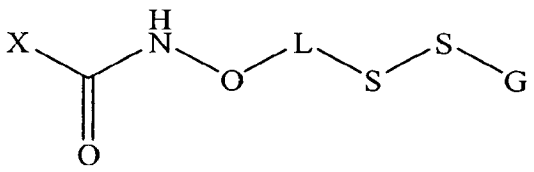
107. The method of claim 92, wherein the oxidant comprises molecular iodine, hydrogen peroxide, an alkyl hydroperoxide, a peroxy acid, a dialkyl sulfoxide, a high valent metal, a metal oxide, or a halogen transfer agent.

108. The method of claim 92, wherein the oxidant comprises a gas comprising oxygen.

109. The method of claim 108, wherein the oxidant further comprises hydrogen peroxide.

110. A compound made by the method of claims 92-109.

111. A compound comprising at least one fragment comprising the formula XVIII



XVIII

wherein

X comprises a residue of a macromolecule;

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof; and

G comprises a residue of a thiolated compound.

112. The compound of claim 111, wherein the macromolecule comprises an oligonucleotide, a nucleic acid or a metabolically stabilized analogue thereof, a polypeptide, a lipid, a glycoprotein, a glycolipid, or a pharmaceutically-acceptable compound.

113. The compound of claim 111, wherein the macromolecule comprises a polysaccharide, a protein, or a synthetic polymer.

114. The compound of claim 111, wherein X comprises a residue of a sulfated-glycosaminoglycan.

115. The compound of claim 111, wherein X comprises a residue of chondroitin, chondroitin sulfate, dermatan, dermatan sulfate, heparin, heparan sulfate, alginic acid, pectin, or carboxymethylcellulose.

116. The compound of claim 111, wherein X is a residue of hyaluronan.

117. The compound of claim 111, wherein G comprises a residue of a thiolated polysaccharide.

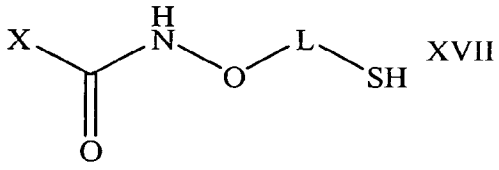
118. The compound of claim 111, wherein G comprises a residue of a thiolated sulfated-glycosaminoglycan.

119. The compound of claim 111, wherein G comprises a residue of thiolated chondroitin sulfate, thiolated dermatan, thiolated heparan, thiolated heparin, thiolated dermatan sulfate, thiolated heparan sulfate, thiolated alginic acid,

thiolated pectin, or thiolated carboxymethylcellulose.

120. The compound of claim 111, wherein G comprises a residue of thiolated hyaluronan.

121. A method for making a compound comprising reacting a first thiolated compound comprising the formula XVIII



XVII

wherein

X comprises a residue of a macromolecule, and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

with at least one electrophilic compound having at least one thiol-reactive electrophilic functional group.

122. The method of claim 121, wherein the electrophilic compound has at least two thiol-reactive electrophilic groups.

123. The method of claim 121, wherein the electrophilic compound has from 2 to 100 thiol-reactive electrophilic groups.

124. The method of claim 121, wherein the macromolecule comprises an oligonucleotide, a nucleic acid or a metabolically stabilized analogue thereof, a polypeptide, a lipid, a glycoprotein, a glycolipid, a polysaccharide, a protein, a synthetic polymer, or a pharmaceutically-acceptable compound.

125. The method of claim 121, wherein the macromolecule comprises a

polysaccharide, wherein the polysaccharide comprises a sulfated-glycosaminoglycan.

126. The method of claim 121, wherein the macromolecule comprises a polysaccharide, wherein the polysaccharide comprises chondroitin sulfate, dermatan, heparan, heparin, dermatan sulfate, heparan sulfate, alginic acid, pectin, or carboxymethylcellulose.

127. The method of claim 121, wherein the macromolecule comprises a polysaccharide, wherein the polysaccharide comprises hyaluronan.

128. The method of claim 121, wherein the macromolecule comprises a protein, wherein the protein comprises an extracellular matrix protein, a partially hydrolyzed extracellular matrix protein, or a chemically-modified extracellular matrix protein.

129. The method of claim 121, wherein the macromolecule comprises a protein, wherein the protein comprises collagen, elastin, decorin, laminin, or fibronectin.

130. The method of claim 121, further comprising reacting a second thiolated compound with the first thiolated compound, the electrophilic compound, or a combination thereof, wherein the first and second compounds are the same or different.

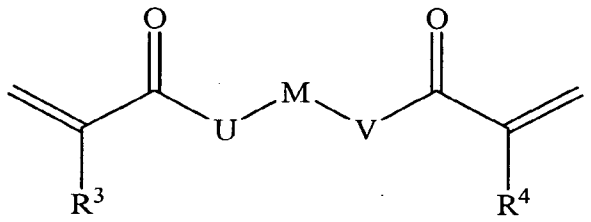
131. The method of claim 121, wherein the thiol-reactive electrophilic functional group comprises an electron-deficient vinyl group.

132. The method of claim 131, wherein the electron-deficient vinyl group comprises a nitro group, a cyano group, an ester group, an aldehyde group, a keto group, a sulfone group, or an amide group.

133. The method of claim 121, wherein the electrophilic compound has two electron-deficient vinyl groups, wherein the two electron-deficient vinyl groups are the same.

134. The method of claim 121, wherein the electrophilic compound comprises a diacrylate, a dimethacrylate, a diacrylamide, a dimethacrylamide, or a combination thereof.

135. The method of claim 121, wherein the electrophilic compound comprises the formula XX



XX

wherein

R^3 and R^4 comprise, independently, hydrogen or lower alkyl;

U and V comprise, independently, O or NR^5, wherein R^5 is, independently, hydrogen or lower alkyl; and

M comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

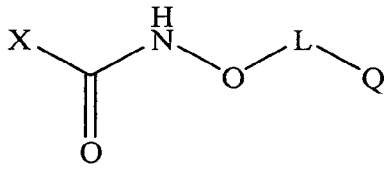
136. The method of claim 135, wherein R^3 and R^4 are hydrogen, U and V are oxygen, and M is a polyether group.

137. The method of claim 135, wherein R^3 and R^4 are hydrogen, U and V are NH, and M is a polyether group.

138. The method of claim 135, wherein R^3 and R^4 are methyl, U and V are oxygen, and M is a polyether group.

139. The method of claim 135, wherein R^3 and R^4 are methyl, U and V are NH, and M is a polyether group.

140. A method for making a compound comprising reacting a first electrophilic macromolecule comprising the formula XVI



XVI

wherein

X comprises a residue of the macromolecule;

Q comprises a thiol-reactive electrophilic functional group; and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof,

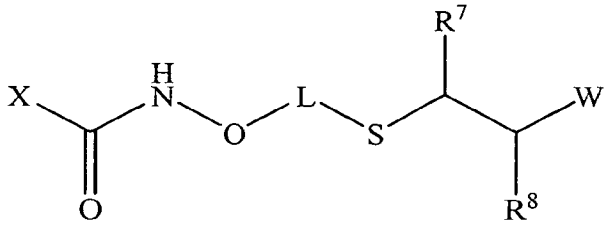
with at least one compound having at least two thiol groups.

141. The method of claim 140, wherein X comprises a residue of a polysaccharide.

142. The method of claim 140, wherein X comprises a residue of hyaluronan.

143. The compound produced by the process of claims 120-142.

144. A compound comprising at least one fragment comprising the formula XXI



XXI

wherein

R^7 and R^8 comprise, independently, hydrogen or lower alkyl;

W comprises an electron-withdrawing group;

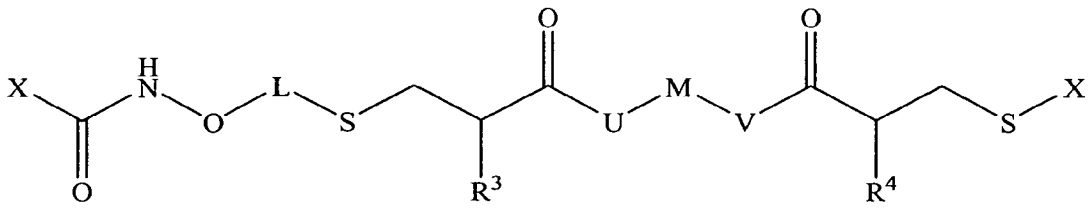
X comprises a residue of a macromolecule; and

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

145. The compound of claim 144, wherein X comprises a polysaccharide residue.

146. The compound of claim 144, wherein W comprises $OC(O)R^{20}$, wherein R^{20} comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, or a combination thereof.

147. A compound comprising at least one fragment comprising the formula XXII



XXII

wherein

R^3 and R^4 comprise, independently, hydrogen or lower alkyl;

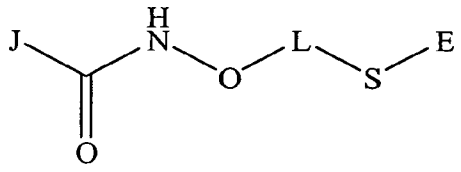
U and V comprise, independently, O or NR^5, wherein R^5 is, independently, hydrogen or lower alkyl;

X comprises a residue of a macromolecule, wherein each residue is the same or different; and

L and M comprise, independently, a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl

group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof.

148. A compound comprising the fragment comprising the formula XXIII



XXIII

wherein

J comprises a protein residue;

L comprises a substituted or unsubstituted hydrocarbyl group, a substituted or unsubstituted heterohydrocarbyl group, a polyalkylene group, a polyether group, a polyamide group, a polyimino group, an aryl group, a polyester, a polythioether group, a polysaccharyl group, or a combination thereof; and

E comprises a fluorescent tag, a radiolabel, a targeting moiety, a lipid, a peptide, a radionuclide chelator with a radionuclide, a spin-label, a PEG camouflage, a metal surface, a glass surface, a plastic surface, or a combination thereof.

149. The compound of claim 148, wherein the protein comprises a naturally-occurring protein or a recombinant protein.

150. The compound of claim 148, wherein the protein comprises an extracellular matrix protein, a partially hydrolyzed extracellular matrix protein, or a chemically-modified extracellular matrix protein.

151. The compound of claim 148, wherein the protein comprises collagen, elastin, decorin, laminin, or fibronectin.

152. A pharmaceutical composition comprising a bioactive agent and the

compound of claims 1-13, 24-44, 48-60, 62-68, 71-75, 77-91, 110-120, or 143-151.

153. A pharmaceutical composition comprising a living cell and the compound of claims 1-13, 24-44, 48-60, 62-68, 71-75, 77-91, 110-120, or 143-151.

154. A method for improving wound healing in a subject in need of such improvement, comprising contacting the wound of the subject with the compound of claims 1-13, 24-44, 48-60, 62-68, 71-75, 77-91, 110-120, or 143-151.

155. A method for delivering at least one bioactive agent to a patient in need of such delivery, comprising contacting at least one tissue capable of receiving the bioactive compound with the composition of claims 152 or 153.

156. A method for delivering living cells to a patient in need of such delivery, comprising contacting at least one tissue capable of receiving the living cells with the composition of claim 152 or 153.

157. The use of the compound of claims 1-13, 24-44, 48-60, 62-68, 71-75, 77-91, 110-120, or 143-151 as a growth factor, an anti-inflammatory agent, an anti-cancer agent, an analgesic, an anti-infection agent, or an anti-cell attachment agent.

158. A composite comprising (1) a first compound comprising a first anti-adhesion compound covalently bonded to a first anti-adhesion support, wherein the first-anti adhesion support comprises any of the compounds in claims 1-13, 24-44, 48-60, 62-68, 71-75, 77-91, 110-120, or 143-151, and (2) a first prohealing compound.

159. The use of the compound of claims 1-13, 24-44, 48-60, 62-68, 71-75, 77-91, 110-120, or 143-151 to repair a tympanic membrane perforation.

160. The use of the compound of claims 1-13, 24-44, 48-60, 62-68, 71-75, 77-91, 110-120, or 143-151 to prevent sinus osteum closure during or after FESS.

161. The use of the compound of claims 1-13, 24-44, 48-60, 62-68, 71-75, 77-91, 110-120, or 143-151 to promote healing after FESS.

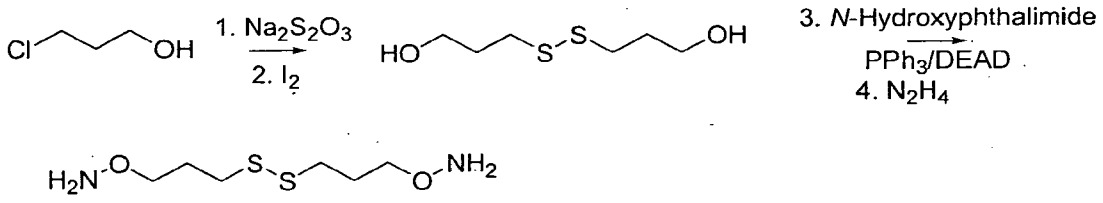
162. The use of the compound of claims 1-13, 24-44, 48-60, 62-68, 71-75, 77-91, 110-120, or 143-151 to reduce scarring after FESS.

163. The use of the compounds of claims 1-13, 24-44, 48-60, 62-68, 71-75, 77-91, 110-120, or 143-151 to prevent adhesion after a surgical procedure.

164. The use of claim 163, wherein the surgical procedure comprises cardiosurgery and articular surgery, abdominal surgery, a surgical procedure performed in the urogenital region, a surgical procedure involving a tendon, laparascopic surgery, pelvic surgery, oncological surgery, sinus and craniofacial surgery, ENT surgery, or a procedure involving spinal dura repair.

ABSTRACT

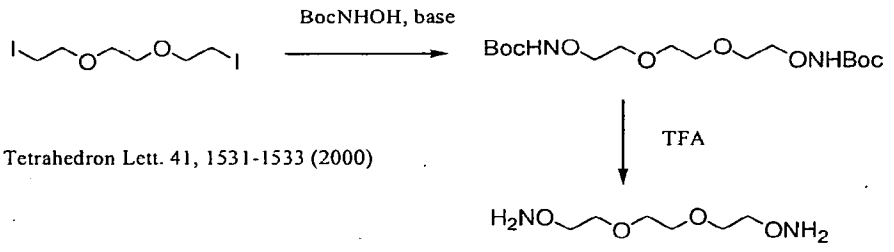
Described herein are compounds such as macromolecules that have been modified in order to facilitate crosslinking. In one aspect, the macromolecule is modified via a condensation reaction between the macromolecule and an aminooxy ether compound, wherein the resultant alkoxyaminated macromolecule can undergo crosslinking with itself or another macromolecule. In another aspect, the macromolecule is modified with a group capable of reacting with a hydrazide compound or an aminooxy ether compound, which will facilitate crosslinking. Also described herein are methods of making and using the modified macromolecules.



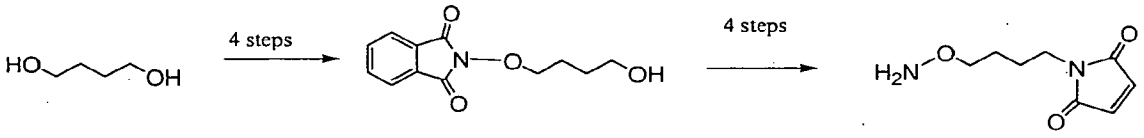
step 1-2, Journal of Organic Chemistry, 55(9), 2580-6; 1990

step 3-4, Journal of the American Chemical Society, 123(31), 7734-7735; 2001

FIGURE 1



Tetrahedron Lett. 41, 1531-1533 (2000)



Bioconj. Chem. 14, 1253-1259 (2003)

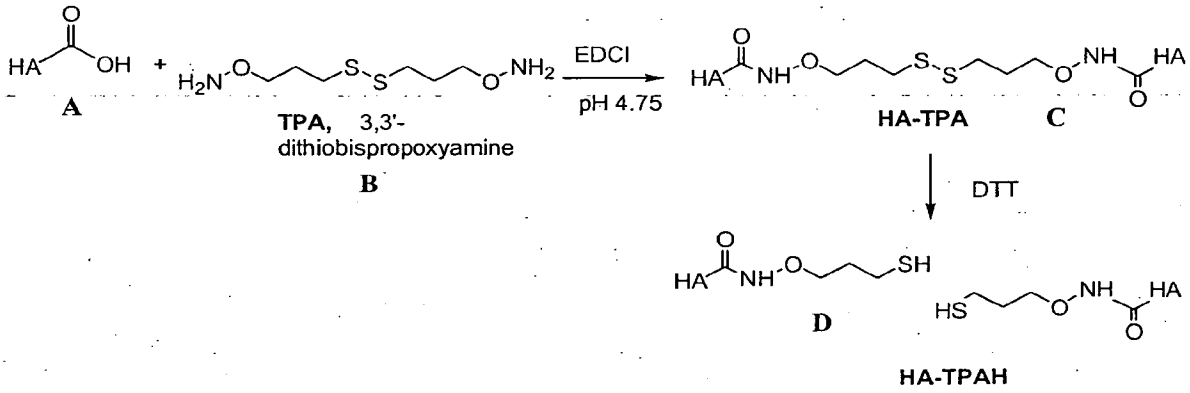


FIGURE 2

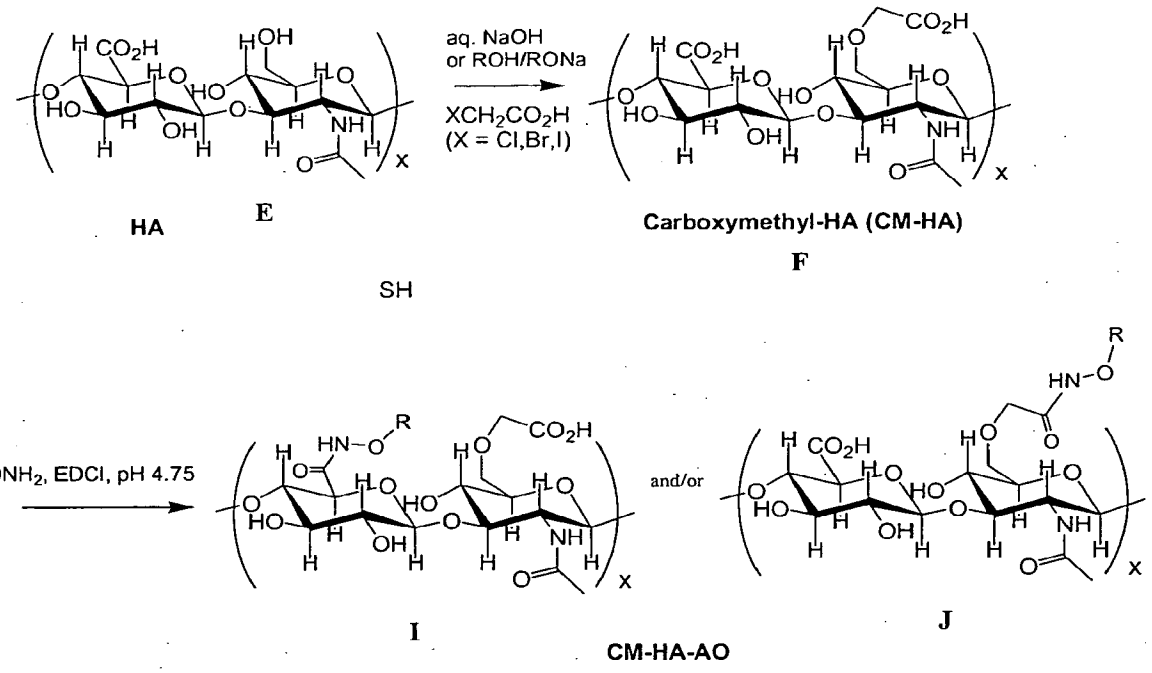

aq. NaOH
or ROH/RONa
XCH2CO2H
(X = Cl,Br,I)
HA
E
Carboxymethyl-HA (CM-HA)
F
SH
RONH2, EDCI, pH 4.75
and/or
I
J
CM-HA-AO

FIGURE 3

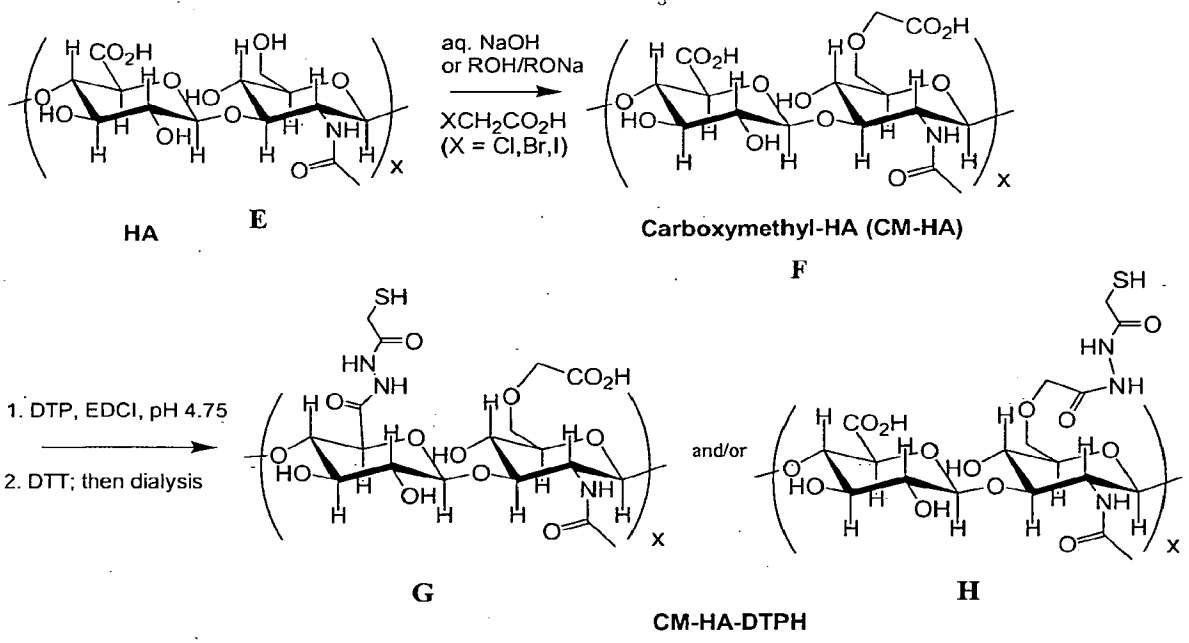

aq. NaOH
or ROH/RONa
XCH2CO2H
(X = Cl,Br,I)
HA
E
Carboxymethyl-HA (CM-HA)
F
1. DTP, EDCI, pH 4.75
2. DTT; then dialysis
and/or
G
H
CM-HA-DTPH

FIGURE 4

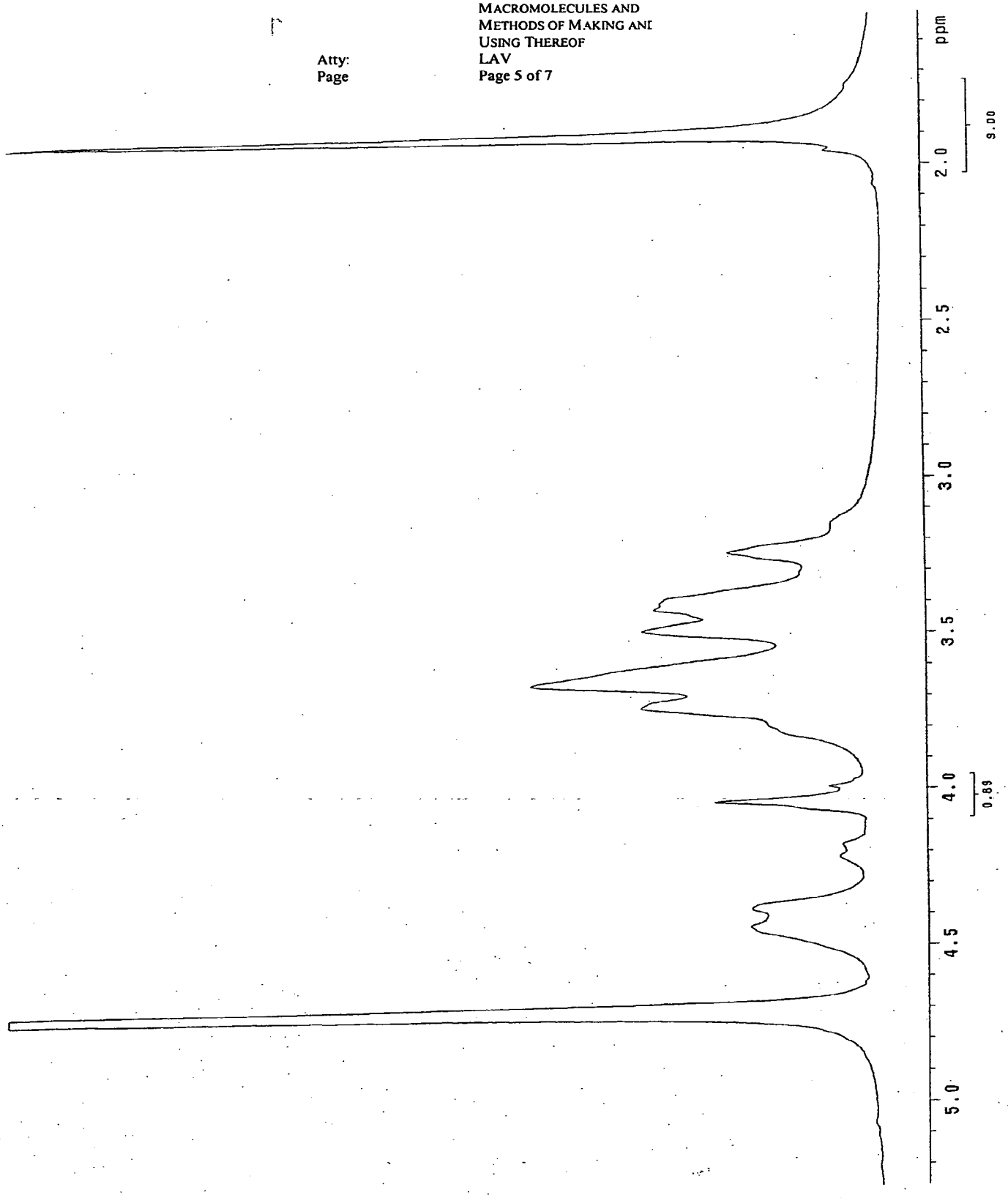


FIGURE 5

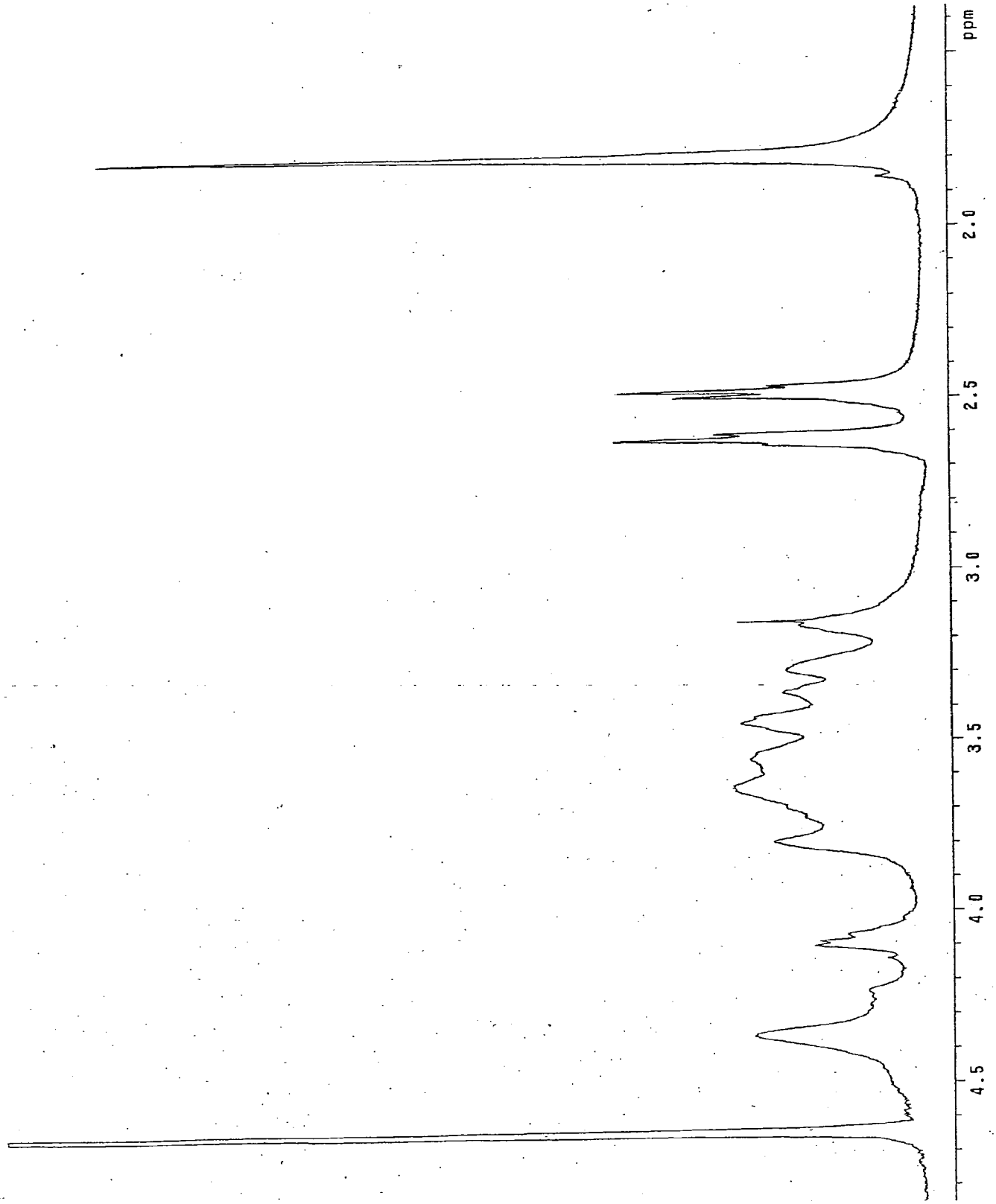


FIGURE 6

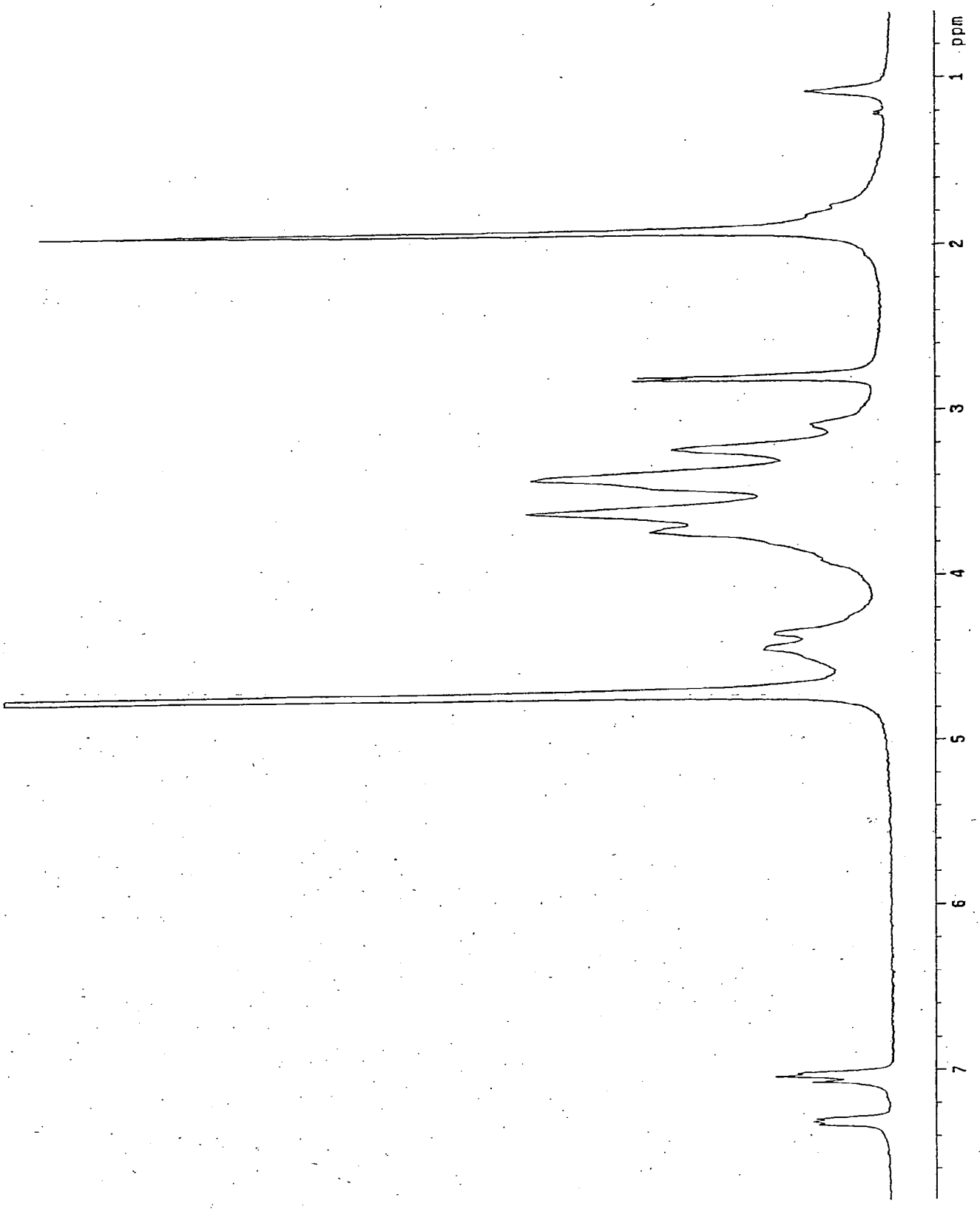


FIGURE 7